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THE BUOYANCY OF CERTAIN NEMATODE EGGS*

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Although floatation technics for the recovery of eggs of parasites from fecal specimens make use of the buoyant force exerted by solutions of a specific gravity higher than that of the eggs, specific gravity values have been reported only for eggs of *Ascaris lumbricoides* (1) and of *Necator americanus* (2). The present study deals with the eggs of the human pinworm (*Enterobius vermicularis*), the human whipworm (*Trichuris trichiura*), the dog whipworm (*Trichuris vulpis*), the dog hookworm (*Ancylostoma caninum*) and fertilized and unfertilized eggs of *Ascaris lumbricoides* from man.

METHODS

Fecal specimens containing eggs of *Enterobius vermicularis, Trichuris trichiura* and *Ascaris lumbricoides* were brought to the laboratory of this Department for routine examination. Feces containing eggs of *Trichuris vulpis* and *Ancylostoma caninum* were passed by naturally infected laboratory dogs.

The fecal samples containing the eggs were thoroughly homogenized in physiologic salt solution (approximately 1 : 50) by a stirring machine (3), which was allowed to run for one hour at 1,600 RPM. The homogenized suspension was strained through one layer of cheesecloth into a gallon jar, which was then filled with physiologic salt solution. Sedimentation was allowed to take place for 6 to 12 hours, usually overnight, following which the supernatant liquid was siphoned off and the sediment containing the concentrated egg suspension kept in the refrigerator at 5° C. The approximate number of eggs present in a measured amount of

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suspension was determined by the centrifugal floatation technic (3), using a 45.6 per cent zinc sulphate solution ($ZnSO_4 + 7H_2O$ U.S.P. granular) of specific gravity 1.250 in a 10 ml Wassermann tube (100×13 mm) and a coverglass resting snugly on the ground top of the tube.

The specific gravities of the eggs were tested by centrifugal floatation in zinc sulphate solutions of graded specific gravities. Equal amounts of suspension containing a known number of eggs were pipetted into each of several Wassermann tubes with ground top. Zinc sulphate solutions of the various specific gravities were then added to fill the tubes so that a superimposed round coverglass contacted the liquid except for a small area occupied by an air bubble. This provided a safeguard against spilling of some of the liquid together with eggs. The preparations were centrifugalized in metal holders with metal sleeves having four fingers projecting upwards to hold the coverglasses in place. An International clinical centrifuge was used with an acceleration period of 20 seconds, a top speed of 2,640 RPM and a deceleration period of 30 seconds. The distance from the axis of the centrifuge to the bottom of the tube was 13.5 cm. After centrifugation for 100 seconds at top speed the coverglasses were rapidly but carefully lifted off each tube, gently rotated manually to mix the material uniformly throughout the adhering drop, and placed drop-down on a vaseline-ringed slide. To the tube from which the coverglass was removed there was added its appropriate zinc sulphate solution until the meniscus reached the rim and a new coverglass was applied. This procedure was repeated again, thus providing 3rd, 4th, and 5th coverglass preparations.

The supernatant liquid remaining after the last preparation had been made was approximately halved by pipetting the upper and lower portions into separate tubes. Zinc sulphate solution of specific gravity 1.250 or 1.300 was added to be sure that the resulting solution exceeded a specific gravity of 1.200. Two to three coverglass preparations were made from each tube.

To the sediment of the original tubes zinc sulphate solution of specific gravity 1.250 was added, the sediment broken up by shaking, and 5 to 7 coverglass preparations were made.

Finally, the sediment of each tube was examined directly by spreading the material on slides to detect eggs which failed to float. The number so found was always less than 1 per cent.

The number of eggs on each coverglass preparation was counted and tabulated. The percentages of recovery from each layer were calculated on the basis of the total number of eggs recovered from each tube. The results show the distribution of the eggs in the surface layer, upper and lower portions of the supernatant liquid, and in the sediment, after centrifugation in zinc sulphate solutions of various specific gravities.

RESULTS

The data in each series of experiments are presented as the percentages of the total number of eggs tested in each solution.

Eggs of the pinworm (*Enterobius vermicularis*).—The specific gravity of pinworm eggs was tested in 3 experiments. In the first two experiments 0.5 ml of the suspension was used, each containing approximately 70 eggs, in the third experiment 0.5 ml containing approximately 90 eggs. The summarized results are presented in Table 1.

TABLE 1.—*Percentages of recovery of pinworm eggs from different levels of $ZnSO_4$ solutions of various specific gravities. Summary of 3 experiments*

Specific gravity	1.080	1.100	1.110	1.115	1.120	1.125	1.180
Surface layer	2	2	6	57	71	85	97
Supernatant liquid upper	0	0	1	6	4	2	0
Supernatant liquid lower	2	2	2	1	1	2	1
Sediment	96	96	91	36	24	11	2

No essential difference was found when fully embryonated pinworm eggs were tested 24 hours after being passed and after 5 days in the refrigerator.

The greater the yield of eggs is on the first coverglass preparation removed from the surface layer, the more efficient is the concentration by centrifugal floatation. For *Enterobius vermicularis* eggs the efficiency percentages of the first coverglass preparations removed from the solutions of the various specific gravities were as follows: specific gravity 1.080, *2;* 1.100, *2;* 1.110, *1;* 1.115, *12;* 1.120, *56;* 1.125, *44* and 1.180, *70.*

Whipworm eggs.—The specific gravity of eggs of the human whipworm (*Trichuris trichiura*) was tested in 5 experiments with no essential difference in the results. The amounts of suspension used were 0.2 ml containing approximately 150 eggs, 0.2 ml with approximately 200 eggs, 0.4 ml with approximately 210 eggs, 0.4 ml with 150 eggs and 1 ml with approximately 300 eggs. The summarized data are presented in Table 2.

TABLE 2.—*Percentages of recovery of* Trichuris trichiura *eggs from different levels of $ZnSO_4$ solutions of various specific gravities. Summary of 5 experiments*

Specific gravity	1.120	1.130	1.140	1.150	1.160	1.165	1.170	1.180	1.200	1.300
Surface layer	0	1	2	23	49	66	75	85	99	100
Supernatant liquid upper	0	0	2	7	5	5	2	5	0	0
Supernatant liquid lower	0	0	4	7	7	5	4	3	0	0
Sediment	100	99	92	63	39	24	19	7	1	0

The efficiency percentages of the first coverglass preparations removed from the surface layers of the various solutions were as follows: specific gravities 1.120 and 1.130, *0;* 1.140, *1;* 1.150, *8;* 1.160, *33;* 1.165, *26;* 1.170, *32;* 1.180, *52;* 1.200, *90;* and 1.300, *93.*

Centrifugation times of 50 and 280 seconds at top speed yielded fewer eggs than did 100 seconds. By employing sodium chloride solutions of specific gravities 1.180 and 1.200 the percentages of eggs buoyed up were

respectively *18* and *73* as compared to *85* and *99* in zinc sulphate solutions of the same specific gravities. The corresponding efficiencies of the first coverglass preparations were *8* and *53* in sodium chloride and *52* and *90* in zinc sulphate solution.

The specific gravity of eggs of the dog whipworm (*Trichuris vulpis*) was tested in 2 experiments. The amounts of suspension used were 0.3 and 0.5 ml containing approximately 80 and 100 eggs respectively. The summarized results are presented in Table 3.

TABLE 3.—*Percentages of recovery of* Trichuris vulpis *eggs from different levels of $ZnSO_4$ solutions of various specific gravities. Summary of 2 experiments*

Specific gravity	1.140	1.150	1.160	1.180	1.200
Surface layer	0	37	88	92	100
Supernatant liquid ..	0	0	0	0	0
Sediment	100	63	12	8	0

Eggs of the dog hookworm (*Ancylostoma caninum*).—The specific gravity of eggs of *Ancylostoma caninum* was tested in 2 experiments. The amounts of suspension used were 0.5 and 0.4 ml containing approximately 200 and 180 eggs respectively. The summarized results are presented in Table 4.

TABLE 4.—*Percentages of recovery of* Ancylostoma caninum *eggs from different levels of $ZnSO_4$ solutions of various specific gravities. Summary of 2 experiments*

Specific gravity	1.040	1.055	1.150	1.180	1.200
Surface layer	0	38	92	100	99
Supernatant liquid ..	1	10	0	0	0
Sediment	99	52	8	0	1

For these *Ancylostoma caninum* eggs the efficiency percentages of the first coverglass preparations removed from the surface layers of the solutions with the various specific gravities were as follows: specific gravity 1.040, *0;* 1.055, *21;* 1.150, *81;* 1.180, *87;* and 1.200, *92.*

Eggs of *Ascaris lumbricoides.*—The specific gravity of fertilized *Ascaris lumbricoides* eggs was tested in 5 experiments. The amounts of suspension used were 0.1, 0.1, 0.1, 0.2, 0.4 ml containing approximately 250, 300, 500, 600 and 450 eggs. The results in these experiments differed considerably; the range in results is presented in Table 5, the

TABLE 5.—*Percentages of recovery of fertilized* Ascaris lumbricoides *eggs from different levels of $ZnSO_4$ solutions of various specific gravities. Range of results in 5 experiments*

Specific gravity	1.080	1.100	1.110	1.120	1.140	1.180	1.200
Surface layer	0	0–1	1–56	25–97	90–99	97–100	98–100
Supernatant ∫ upper ..	0	0	0– 6	5– 0	2– 0	0	0
liquid { lower ..	0	0	0– 4	4– 0	0	0	0
Sediment	100	100–99	99–34	66– 3	8– 1	3– 0	2– 0

lower percentages being obtained with 500 eggs in each test tube, the higher ones with 300 eggs.

For these fertilized *Ascaris* eggs the efficiency percentages of the first coverglass preparations removed from the surface layers of the solutions with the various specific gravities ranged as follows: specific gravities 1.080 and 1.100, *0;* 1.110, *1* to *32;* 1.120, *6* to *73;* 1.140, *53* to *71;* 1.180, *57* to *79* and 1.200, *94.* A top speed centrifugation time of 50 and 280 seconds did not essentially alter the results.

The specific gravity of unfertilized eggs of *Ascaris lumbricoides* was tested in 2 experiments. The amounts of suspension used were 0.2 and 0.4 ml containing approximately 90 and 120 eggs. The summarized results are presented in Table 6.

TABLE 6.—*Percentages of recovery of unfertilized* Ascaris lumbricoides *eggs from different levels of* $ZnSO_4$ *solutions of various specific gravities. Summary of 2 experiments*

Specific gravity	1.160	1.180	1.200	1.250
Surface layer	0	3	39	99
Supernatant liquid	0	2	13	0
Sediment	100	95	48	1

DISCUSSION

The specific gravities of eggs of the pinworm, the whipworm, the hookworm and *Ascaris* were tested in zinc sulphate solutions of various specific gravities by centrifugal floatation. Zinc sulphate and centrifugal floatation were chosen in order to obtain additional quantitative evaluation of the zinc sulphate centrifugal floatation technic (3, 4). Buoyancy tests are used for the determination of specific gravity values. However, the difference in yield of whipworm eggs from sodium chloride and zinc sulphate solutions of the same specific gravity adds evidence that the specific gravity of a biologic object determined by its buoyancy is not necessarily identical with its true specific gravity, due to the possible interaction of floating medium and object (2). The zinc sulphate medium apparently interferes less, possibly by virtue of the difficulty of the sulphate ion to penetrate the living cell membrane (5).

For the floatation technic a solution of a specific gravity equal to that of the eggs is not sufficient. The specific gravity of the floating medium must be higher. The difference in the two values influences the number of eggs buoyed up and especially the number recovered from the first coverglass preparation. A centrifugation time of 100 seconds top speed was employed after it was found that 50 and 280 seconds provided lower yields on the first coverglass preparation. However, it is important to note that the manner in which the superimposed coverglass is lifted from the tubes influences the yield, as emphasized by Lane (6).

While few if any eggs of *Enterobius vermicularis* floated in solutions of specific gravities of 1.110 and lower, in a solution of 1.115 the eggs were distributed in the surface layer, the supernatant liquid and the sedi-

ment. In solutions of higher specific gravity the top layer yield increased. Thus, the specific gravity of *Enterobius vermicularis* eggs, as determined by buoyancy tests in zinc sulphate solutions, is approximately 1.115. For floatation technics a solution of this specific gravity obviously does not provide a sufficient yield. Only 57 per cent of the total number of eggs was found in the surface layer, and only 12 per cent on the first coverglass preparation. While a solution of the specific gravity 1.180 will float almost all of the *Enterobius vermicularis* eggs, the first coverglass preparation yielded little more than two-thirds of the total number.

In solutions of specific gravity 1.140 and lower only an occasional egg of *Trichuris trichiura* or *Trichuris vulpis* floated; in solutions with specific gravities 1.150 and 1.160 the eggs were distributed in the surface layer, the supernatant liquid and the sediment. Thus, the specific gravity of *T. trichiura* eggs, as determined by buoyancy tests, appears to be between 1.150 and 1.160.

Solutions of higher specific gravity have to be used for concentration of these eggs. In a solution of specific gravity 1.180, 85 per cent of the total number was recovered from the surface layer by consecutive coverglass preparations, although the first preparation yielded only one half of the total number present. A first coverglass yield of 90 per cent was obtained with a solution of specific gravity 1.200. These yields are in accordance with results obtained by Faust et al. in a qualitative study (4), in which more than 10 per cent of infections actually present were missed, when centrifugal floatation in zinc sulphate solution of specific gravity 1.180 was employed. The specific gravity values of *T. vulpis* eggs were similar to those of *T. trichiura.*

Lane (6) found a sodium chloride solution of specific gravity 1.150 inadequate for floating whipworm eggs that had been preserved under water for more than 4 weeks. A sodium chloride solution of specific gravity 1.200 floated approximately one-half the number that floated in calcium chloride solutions of specific gravity 1.300 and higher. These findings indicate that old whipworm eggs in these salt solutions need a greater buoyant force than do fresh eggs in zinc sulphate solutions.

Eggs of *Ancylostoma caninum* were found distributed in the surface layer, the supernatant liquid and the sediment in zinc sulphate solutions of specific gravity 1.055, thus suggesting that this is their specific gravity. This value agrees exactly with that of *Necator americanus* eggs previously reported (2). While in solutions of specific gravity 1.150 more than 90 per cent of *A. caninum* eggs was recovered from consecutive preparations removed from the surface layer, the efficiency of the first coverglass preparation was 80 per cent. Heavier solutions were still more efficient, solutions of specific gravity 1.180 yielding 87 per cent.

Lane (7) has stressed the need of using a solution having at least a

specific gravity of 1.150 for the practical purpose of concentrating human hookworm eggs by floatation. The data presented for *A. caninum* are in agreement with Lane's findings. They are further substantiated by the following unpublished results obtained during the study of the specific gravity of *Necator americanus* eggs (2) : The efficiency of the first coverglass preparations from the surface layers of solutions of specific gravities 1.045 and 1.050 was 0; of 1.055, 4 per cent; of 1.060, 53 per cent; of 1.065, 56 per cent and 1.180, 98 per cent.

Fertilized eggs of *Ascaris lumbricoides* in the one-cell stage varied in their specific gravity values. In one series of experiments the eggs were buoyed up in solutions of specific gravity 1.120 to 1.130 and in another, of 1.110, so that 1.110 to 1.130 may be considered the range. On the first coverglass preparation, when zinc sulphate solution of specific gravity 1.180 was used, only 57 to 79 per cent of the total yield was recovered, while a solution of specific gravity 1.200 yielded 94 per cent. Unfertilized eggs of *Ascaris* floated in solutions of specific gravity 1.200, but more readily in solutions of specific gravity 1.250.

Lane (6) found that only 4 per cent of fertilized *Ascaris* eggs floated in a sodium chloride solution of specific gravity 1.150, while in a solution of specific gravity 1.200, about one half the number floated as with a calcium chloride solution of specific gravity 1.300. However, these results were obtained with eggs more than 4 weeks after being passed and in solutions other than zinc sulphate. In 1926, Sasaki (1) reported the specific gravity of fertilized *Ascaris* eggs as varying between 1.083 and 1.187, the less mature eggs being heavier than the more mature ones, unfertilized eggs being still lighter. The difference between Sasaki's findings and those reported here might be due to different floating media employed. The information necessary for comparison was not available, since the report is written in Japanese.

SUMMARY

The specific gravities of several nematode eggs have been tested in zinc sulphate solutions of various specific gravities. However, values thus determined are not necessarily the actual specific gravities. For the concentration of these eggs by centrifugal floatation a solution of a specific gravity higher than their own is necessary. The centrifugation time is a function, mathematically expressed, of the difference between the specific gravities of the eggs and the solution.

Enterobius vermicularis eggs were buoyed up in solutions of specific gravity 1.115 and higher. A solution of specific gravity 1.180 yielded 97 per cent of the total in the surface layer and 70 per cent on the first coverglass preparation.

Trichuris trichiura eggs were buoyed up in solutions of specific grav-

ity 1.150 and higher. A solution of specific gravity 1.180 yielded 85 per cent of the total in the surface layer and 52 per cent on the first coverglass preparation, while a solution of specific gravity 1.200 yielded 99 and 90 per cent respectively.

Trichuris vulpis eggs were buoyed up in solutions of specific gravity 1.150 and higher.

Ancylostoma caninum eggs were buoyed up in solutions of specific gravity 1.055 and higher. A solution of specific gravity 1.150 floated 92 per cent of the eggs and a solution of specific gravity 1.180, 100 per cent, with an efficiency of the first coverglass preparation of 87 per cent.

Fertilized *Ascaris lumbricoides* eggs differed in their buoyancy in different experiments within the range 1.110 to 1.130. A solution of specific gravity 1.180 floated almost all of these eggs, while the efficiency of the first coverglass preparation varied between 57 and 79 per cent. A solution of specific gravity 1.200 yielded 94 per cent on the first coverglass preparation.

Only 39 per cent of unfertilized *Ascaris lumbricoides* eggs was buoyed up in solutions of specific gravity 1.200, while a specific gravity of 1.250 yielded 99 per cent.

The data on the specific gravity of these eggs provide basic information on the required specific gravity of the solution to be employed for floatation technics. The differences in the specific gravities of the eggs examined suggest the possibility of differential floatation.

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STUDIES ON DERMATITIS-PRODUCING SCHISTOSOMES IN EASTERN MASSACHUSETTS, WITH EMPHASIS ON THE STATUS OF *SCHISTOSOMATIUM PATHLOCOPTICUM* TANABE, 1923[1]

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During the summer of 1940 it was possible to study the potential or real schistosome dermatitis problems which occur in eastern Massachusetts. The center of operations was the Harvard Medical School at Boston, where Tanabe, early worker on rodent schistosomes in the United States, had studied. Not only was the prevalence of the causative organisms in the region investigated but five of Tanabe's original slides of *Schistosomatium pathlocopticum* were studied.

HISTORICAL

Tanabe (1923) infected white mice and white rats with a schistosome cercaria found in *Stagnicola palustris* collected in the vicinity of Boston, Massachusetts, primarily from the Back Bay Fens region. Because the adult worms were different from any previously described, he erected a new genus and species, *Schistosomatium pathlocopticum,* for his specimens. This was the first schistosome life cycle to be completed experimentally in this country. He was unable however, to demonstrate a natural host for the worm.

Price (1929) described a new adult schistosome obtained experimentally in laboratory rats and mice. She produced the infection by experimental exposures to *Cercaria douthitti* Cort, 1914, from the snails, *Lymnaea stagnalis apressa* Say and *S. palustris* (Müller). Later Price (1931) reported quite fully the entire life cycle and believed field mice to be the natural hosts of the fluke. She considered the worm to be very closely related to *S. pathlocopticum* but because of certain differences she discovered in her study, did not believe them synonyms. Price was able to infect *Peromyscus maniculatus* (Wagner) Bangs, *Mus musculus* Linnaeus, *Microtus pennsylvanicus* (Ord.), white mice, white

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rats, and a cat. Her examinations of several species of mammals from nature were negative with the exception of two meadow mice, one of which had one pair of worms in a mesenteric vein of the cecum while the other had three males and one female in a similar position. The animals were collected in the region of Third Sister Lake, near Ann Arbor, Michigan.

The above were the only records of natural infections until Penner (1938) tentatively determined as *Schistosomatium douthitti* (Cort) worms found in 32 out of 330 muskrats from the vicinity of Minneapolis, Minnesota, examined during March and April, 1938, and in one of two muskrats examined at the University of Michigan Biological Station during August, 1938. Since that time Penner (1939, 1940) has experimentally infected muskrat, guinea pig, snow-shoe hare, and cottontail and laboratory rabbits with *C. douthitti* and has also collected data on the role played by the muskrat as the natural definitive host. Since the spring of 1938, 374 muskrats in the vicinity of Minneapolis, have been examined for schistosomes. Forty-two (11.2%) were found infected. One of two brown rats examined November 6, 1939, had in the liver spineless schistosome eggs which were identified as those of *S. douthitti*. The rat was caught in water about two feet from shore in a baited live trap. Most of the eggs were walled off in the liver with fibrous tissue and no eggs were found in the feces of the rat. Mr. A. C. Cuckler, in a recent personal communication, reported finding schistosomes in *Microtus* trapped during July, 1940, at a pond near Edina, Minnesota. Although they have not been studied, the worms are likely *S. douthitti,* as extensive snail collecting in the pond has failed to reveal any species of schistosome cercariae other than *C. douthitti.*

Porter (1938) reported *Schistosomatium pathlocopticum* in experimental mice at Johannesburg, South Africa. She exposed a month old mouse to cercariae from *Bulinus tropicus* for 45 minutes and at the death of the mouse in 32 days, found one male and one female worm which agreed in many particulars with the adults found by Tanabe (1923). However, her measurements, especially of suckers, are somewhat at variance with measurements of either *S. pathlocopticum* as described by Tanabe, or *S. douthitti.* The material with which she worked was limited and it is possible that she was dealing with an entirely different species of rodent schistosome.

McLeod (1940) recorded the examination of 460 snow-shoe hares (*Lepus americanus*), 240 gophers (*Citellus,* 3 spp.), and 36 muskrats in the laboratories of the University of Manitoba but all were negative for schistosomes. Other examinations of rodents in this country have failed to reveal schistosomes.

THE STATUS OF *Schistosomatium pathlocopticum* TANABE, 1923

One of the main purposes of the study of dermatitis-producing schistosome cercariae in the vicinity of Boston was to determine the relationship of Tanabe's species, *S. pathlocopticum,* to *S. douthitti.* Extensive collecting failed to reveal any schistosome cercariae which could be considered identical with the cercariae for either species as described. Therefore, before going further with the problem of schistosome dermatitis in Massachusetts it is deemed advisable to discuss the status of *S. pathlocopticum* as determined from Tanabe's publications and from some of his preserved material consisting of five slides labelled as follows: C. B. Cercaria; Male from mesenteric v. of mouse 33 days after infection July 7, 1922; ♀ from mes. vein of ms. 25 days after infection July 15, '22; Intestine of Ms. B; and Eggs from Liver of Ms. G. July 21, 1922. From a thorough study of the slides and of the results from certain experiments I have come to the conclusion that Tanabe's species is a synonym of *S. douthitti* (Cort, 1914). The evidence to warrant the above conclusion is incorporated in the following discussion.

ADULT WORMS

Tanabe reported the maximum length of the males of *S. pathlocopticum* to be 11.8 mm and that of the females, 10.2 mm. The age of the infection was not given. These measurements are considerably longer than those given by other authors for specimens of *Schistosomatium.* Penner (1940) found the maximum length of males of *S. douthitti* in white mice to be 7.14 mm, and that of the females, 4.53 mm. This in itself, however, is not sufficient reason for separating it from *S. douthitti* as there may be several other causes of length difference, as for example:

a. Distortion of the specimens on extraction from host. I have been able to extend specimens of *S. douthitti* to more than 10 mm when extracting them from the mesenteric vessels of mice dead several hours before autopsy. It is suggested that Tanabe's longest worms may have been removed when dead.

b. Intensity of infection affects the size of the worm (Price, 1931). In my experiments the largest worms have generally been found in lightly infected mice.

c. That there may be remarkable differences in the size of worms has been recognized for other species of schistosomes. Cort (1923) found quite a range of variation in specimens of *S. japonicum* recovered on any given day after infection. In general, the same has been found true of *S. douthitti* (Penner, 1940).

d. The length of time the infection has been allowed to run before the host is autopsied. Cort (1921) in studying *S. japonicum* noted remark-

able changes in size as the worms passed from the cercarial to the adult stage, the worms increasing as much as 130 times their cercarial size before becoming mature adults. Specimens of *S. japonicum* from mice, although usually smaller, have reached the size of 8 mm for males and 11 mm for females from hosts examined 62 days after exposure (Katsurada, 1914).

e. In addition, the age of the host, the strain of laboratory animal used, individual resistance or susceptibility of the host to infection, and diet of the host are among the factors which may influence the size of the worm.

By experimentally infecting mice with cercariae of *S. douthitti* and performing autopsies at lengths of time corresponding to those of Tanabe's infections the author was able to obtain comparable measurements (Table 1).

TABLE 1.—*Comparative maximum measurements, in millimeters, of male* Schistomatium *taken from experimental infections of white mice*

Days	Tanabe (1923) Cercariae from *S. palustris*	Penner (present paper)
12	1.658	1.20 (cercariae from *L. stagnalis*)
21	3.85	3.84 (cercariae from *S. palustris*)
24	5.785	
27		5.04 (cercariae from *L. stagnalis*)
30	6.338	7.14 (cercariae from *L. stagnalis*)
	Maximum measurements of females	
21	3.851	3.18 (cercariae from *S. palustris*)

In my experimental infections the mice were exposed by being placed in a bell jar containing water with cercariae. The time of exposure varied from 4–20 minutes depending on the number of cercariae used. The water was deep enough so the feet and lower parts of the mice were under water.

In Tanabe's mounted material which I have had the opportunity of studying, there are two slides of adult worms, one containing two males from a 33 day infection in mice, and the other containing three females from a 25 day infection in mice. The specimens were apparently mounted in balsam and ringed with some kind of sealing cement. The mounting medium has partially evaporated thereby distorting the specimens to a certain degree. They are, however, still satisfactory for use in observing general morphological features.

In order to have *S. douthitti* specimens of the same age, mice were infected with known *S. douthitti* cercariae and autopsied at dates corresponding to those on Tanabe's slides. Measurements are indicated in Table 2.

The measurements of Tanabe's specimens are for the most part in agreement with those given for *S. douthitti.* Such differences as do exist, can be accounted for by the excessive flattening caused by the drying of his slides over a period of several years.

TABLE 2.—*Measurements, in millimeters, of Tanabe's mounted specimens as compared with those of known* S. douthitti *adults of the same ages*

♂♂	33 days Tanabe (2 specimens)	33 days Penner (10 specimens)*
Body length	5.7 –7.14	3.4 –7.12
Length forebody	2.7 –3.14	1.4 –2.4
Width forebody	0.43–0.56	0.36–0.60
Length hindbody	3.97–3.99	1.74–4.72
Width hindbody	0.61–0.65	0.21–0.54
Length oral sucker	0.16–0.16	0.12–0.18
Length ventral sucker	0.14–0.22	0.18–0.20
Oral s. to ventral s.	0.96–1.11	0.57–0.84
♀♀	25 days Tanabe (3 specimens)	25 days Penner (10 specimens)*
Body length	4.5 –6.3	1.92–4.50
Body width	0.18–0.31	0.15–0.30
Oral s. length	0.09–0.14	0.09–0.11
Ventral s. length	0.14–0.18	0.09–0.13
Oral s. to ventral s.	0.41–0.45	0.30–0.42

* Snail host, *Stagnicola reflexa.*

Another character which has been used to separate *S. pathlocopticum* from *S. douthitti* has been testicular lobation. Tanabe's reports for *S. pathlocopticum* were 14–16 follicles and Price (1931) for *S. douthitti* 15–36. However, the two males in Tanabe's material had 20 and 22 distinct follicles with several smaller lobules which are not so distinct. The testes of a 33 day old male *S. douthitti* from an experimental infection in white mouse are quite similar. Tanabe apparently erred in counting the testicular lobation in his own experimental material. I have found all gradations of testicular lobation in experimental material from a lobed mass to as many as 29 follicles. The situation seems to depend largely on the duration of the infection. For example, as few as 15 lobes have been counted in a 21 day infection and as many as 29 in a 130 day infection in laboratory mice.

It is interesting to note in this respect that Porter (1938) in reporting *S. pathlocopticum* from South Africa, figured and indicated that the male worm she studied, had 14 distinct testicular lobules. If the lobation is found constant after more material from South Africa is studied, it may prove significant in determining the status of Porter's specimens. It is possible, as mentioned before in this paper, that, provided the adult and cercarial morphology are as she described them, she was dealing with a new species.

EGG

The section of liver in Tanabe's material marked "Eggs from Liver of Ms. G, July 21, 1922," is not very satisfactory for measuring the eggs. A series of 10 ranged from 45–62 microns in length and 42–50 microns in width. Table 3 gives comparative measurements of *Schistosomatium* eggs as recorded by different authors. It also indicates the variation in size of eggs as they are found in different habitats. The

TABLE 3.—*Comparative measurements, in microns, of* Schistosomatium *eggs as recorded by different authors*

Location of eggs	Tanabe, 1923 10 eggs (mouse)	Price, 1931 50 eggs (mouse)	Penner, present paper 50 eggs (host-muskrat)
Uterus of female worm..	40– 59 long	42– 80 × 30–58	35– 90 × 30–65
Liver of definitive host..	56– 98 × 43–70		50–106 × 40–76
Intestinal wall	49– 75 × 56–87		44–110 × 54–90
Mature or developed egg	80–126 × 58–87	94–122 × 74–98	90–128 × 64–96

developed eggs were found by Price (1931) to occur 28–30 days after the definitive host was exposed to infection. The measurements do not indicate that the material studied might be of more than one species of fluke.

CERCARIA

Tanabe (1923) indicated the close resemblance of the cercaria of *S. pathlocopticum* to *C. douthitti.* The major difference was in the recorded number of penetration glands. Tanabe reported three pairs present in *S. pathlocopticum,* this number being verified, perhaps erroneously, by Porter (1938). Cort (1914) first described *C. douthitti* as having 4 pairs of penetration glands, but Cort (1917) and others have since observed 5 pairs, while Price (1931) added an extra pair of escape glands present in the cercaria before emergence from the daughter sporocyst. Miller (1936) in his key to the Illinois cercariae still retained Cort's (1914) first observation that four penetration glands are present on each side of the body in *C. douthitti.*

Tanabe's slide labelled "C.B. Cercaria" contains 7 cercariae, five of which are considerably flattened and more or less separated from the mounting fluid while the other two are but slightly distorted and in the mounting medium. Several of the cercariae show what appear to be 5 pairs of glands with ducts leading away from them.

After examining this material, I am convinced that Tanabe's material has 5 pairs of penetration glands and feel that he erred in determining the actual number present. Porter (1938) perhaps erred in her study of cercariae which she determined to be *S. pathlocopticum.* Although she reported only 3 pairs of penetration glands, it is quite possible that she did not have enough available material to correctly determine the actual number of glands. Her drawings resemble those published by Tanabe (1923). If there are only 3 pairs of penetration glands in her material, she is dealing with a new species of schistosome worm.

There are 5 or 6 penetration spines on each side of the concavity of the head organ near the openings of the ducts from the penetration glands. Tanabe (1923) described distinct anterior spines on his cercaria, believing their presence signified a difference between the cercariae

of *S. pathlocopticum* and *S. douthitti.* As *C. douthitti* has such spines, this was not a valid difference.

C. douthitti was first described without cuticular spines (Cort, 1914). Later Cort (1917) restudied the species and observed their presence under high power of the microscope. Tanabe (1923) recorded minute spines covering the whole surface of the tail and body in the material he studied and other workers have demonstrated the same for *C. douthitti.*

The flame cell pattern as given by Cort (1917) and Price (1931) is the same as that given by Tanabe (1923). There are 5 pairs of body flame cells and 1 pair in the tail. Porter (1938) erroneously cited the

TABLE 4.—*Comparative measurements, in millimeters, of* Schistosomatium *cercariae as given by different authors*

	Cort, 1914, 1917, mounted	Tanabe, 1923, 10 specimens, 5% formalin	Penner, present paper, 7 of Tanabe's mounted specimens	Price, 1931, 25 specimens, 10% formalin	Porter, 1938, 10 specimens, living	Penner, present paper, 20 specimens, 10% formalin. Cercariae from *L. stagnalis*	Penner, present paper, 20 specimens, 10% formalin. Cercariae from *Stagnicola reflexa* (Say)
Av. body length	.190	.18	.194 (.15–.23)	.204 (.186–.24)	(.115–.18)	.200 (.188–.240)	.195 (.186–.228)
Av. body width	.067	.08	.091 (.06–.10)	.068 (.057–.081)	(.068–.082)	.072	.074
Av. tail stem length	.220	.23	.170 (.14–.18)	.210 (.186–.234)	(.133–.220)	.215	.221
Av. tail stem width	.025	(basal) .045	.035 (.17–.46)	.025	(.036–.043)	.025	.025
Furcal rami length	.089	.10	.064 (.038–.154)	.096 (.08–.11)	(.06–.103)	.092	.095
Head organ length	.057	.05	.059 (.38–.74)	.076 (.067–.082)	(.042–.045)	.071	.070
Head organ width	.045	.047	.062 (.38–.77)	.048 (.038–.062)		.046	.047
Acetabulum diameter	.025	.024	.028 (.02–.035)	.025 (.019–.028)	.03	.025	.025
Eyespots	.007	.008	.008 (.007–.009)	.007 (.006–.009)		.007	.007 (.006–.009)
Pairs penetration glands	4 (1914) 5 (1917)	3	5	5 (6)	3	5	5 (6)
Pairs flame cells	6 (1917)	6	6	6	6	6	6
Anterior spines	Not desc. (1914) Desc. (1917)	Present	Present	Present	Present	Present	Present

cercariae of *S. douthitti* as having 10 flame cells on each side of the body rather than 5, and used this as a difference between *S. pathlocopticum* and *S. douthitti.*

In general Tanabe's observations agree with Price's very complete description of the cercaria of *S. douthitti.* Measurements and other fundamental data are such as to indicate that Tanabe's cercaria is *C. douthitti.* Size, eyespots, spination, etc. are all in agreement with *C. douthitti* of other authors. In order to indicate the essentials without elaborate discussion Table 4 incorporates comparative measurements of *Schistosomatium* cercariae as given by different authors and my measurements of Tanabe's slide material.

From Table 4 it can be discerned that the measurements of Tanabe's mounted specimens and Tanabe's own personal measurements are such as to conform to those given for the cercariae of *S. douthitti.* In Table 5

TABLE 5.—*Measurements, in microns, of Tanabe's permanent cercarial mounts*

	1	2	3	4	5	6	7
Body length	193	192	228	217	224	154	151
Body width	105	98	105	102	105	63	60
Length head organ	60	59	74	70	73	39	38
Width head organ.....	68	70	70	70	77	42	38
Tail stem length	172	140	175	154	178	182	178
Tail stem width.....	35	39	38	42	46	17	25
Furcal rami length....	52 & 56	38 & 42	59 & 68	67 & 70	91 & 154	49 & 53	46
Furcal rami width	14	14	21	21	21	7	7
Ventral sucker length..	14	17	28	28	28	21	21
Ventral sucker width ..	21	21	32	35	35	28	28
Eyespots	8	9	8	8	8	8	7
Penetration glands	5 pr?	5 pr?	5 pr?	5 pr?	5 pr	?	?
Penetration gland ducts	5?	5?	?	?	?	5?	?

the measurements in microns were used in determining the averages of the mounted cercariae on Tanabe's permanent mount of the cercariae. The last two columns are measurements of the specimens not excessively flattened and still in the mounting fluid on the slide. The first five columns include the somewhat flattened specimens.

The only other cercaria which may be placed in very close proximity with the *Schistosomatium* material of North America and South Africa is *Cercaria "C"* Kemp, 1921, knowledge of which is confined to data from preserved material. The latter material from India was placed in Miller's (1924) apharyngeal brevifurcate distome cercariae Group C (*douthitti* of Sewell, in part), along with *C. douthitti* and the cercaria of *S. pathlocopticum.*

Emergence of cercariae from the snail host. Tanabe (1923) observed great numbers of the cercariae of *S. pathlocopticum* discharged daily from infected snails into the surrounding water "—especially on cloudy days and nights, or in clear, recently renewed water." My observations and the observations of other authors on *C. douthitti* fit the observations of Tanabe in these respects. For example Penner (1940)

found that on one very dark day, cercariae of *S. douthitti* from one *Stagnicola reflexa* (Say) emerged at 3:30 PM, whereas on other fair days, emergence occurred from 5:30 to 9:30 PM. Cort and Talbot (1936) found that cercariae of *S. douthitti* began to escape from their snail hosts from 8:30 to 9:30 PM and that almost all that would escape during a 24 hour period would be out within an hour.

Behavior of the cercariae. According to Tanabe, not nearly such a large proportion of the cercariae of *S. pathlocopticum* as of *S. douthitti* remain attached to the surface film during their free life. *C. douthitti* has been found by many authors to spend most of its free life attached to the surface film of the water; nevertheless, on studying emergence, I have found *C. douthitti* free in the water, especially if the bottle is handled or jarred, although invariably the cercariae are later attracted to the surface film. It is quite a common occurrence to note the cercariae free in the water, especially after a change of water on the infected snail. These observations on *C. douthitti* are quite in agreement with Tanabe's observations on what he believed to be a new species.

RESULTS OF INVESTIGATIONS ON THE PRESENCE OF DERMATITIS-PRODUCING SCHISTOSOME CERCARIAE IN EASTERN MASSACHUSETTS DURING THE SUMMER OF 1940

As the cercariae of *S. douthitti* are known to produce schistosome dermatitis or water itch, and Tanabe's species is really *S. douthitti,* eastern Massachusetts already has one schistosome cercaria which can produce water itch. Although other schistosome cercariae have been reported from Massachusetts (Rankin, 1939) none is known to be a dermatitis producer.

Since the main purpose of my study of dermatitis-producing schistosomes in eastern Massachusetts was to straighten out Tanabe's material, it was considered advisable to collect fresh-water snails in areas reported by Tanabe to contain schistosome infected snails. Although Tanabe indicated that his collections were made solely in the Back Bay Fens of Boston, Mr. W. J. Clench, Curator of the Museum of Comparative Zoology at Harvard University, informed me that Tanabe made collections in different parts of eastern Massachusetts other than in the Back Bay Fens region. Because of this, my collections were extended to include accessible waterways that Tanabe might have reached, as well as to include lakes and other areas where potential or real dermatitis problems might prevail. Likewise, collections were made in areas where *Stagnicola palustris* (Müller) had been reported. This information was obtained from the distribution data of the Peabody Museum of Comparative Zoology Mollusc Collection at Harvard University. Information on the distribution of other snails which might be hosts of schistosome cercariae, was also obtained from these records.

Collections were made in various areas arbitrarily designated as follows: Muddy River and Charles River Basin Area (Jamaica Pond through Ward's Pond, Leverett Pond to the Charles River), Hammond's Pond Area (Hammond's Pond, Chestnut Hill Reservoir, Lost Pond), Dedham Area (Charles River two miles on either side of Dedham, Motley Pond, Wigwam Pond, and Mother Brook), Newton Area (Newton, Newton Center, Newtonville, Charles River near Newton Upper Falls and Newton Lower Falls, and West Newton), Cambridge Area (Fresh Pond, Perch Pond, Little River, portion of Alewife Brook and several clay pits), Arlington and West Medford Area (Little Spy Pond, Spy Pond, lower portion of Alewife Brook, Upper and Lower Mystic Lakes), Wakefield Area (Crystal Lake, Lake Quannapowitt, a portion of Saugus and Mill Rivers, and Dark Meadows), Wellesley Area (Lake Waban, Morse's Pond, Jenning's Pond, and None Such Pond), Auburndale Area (Weston and Cambridge Reservoirs, Charles River from Auburndale to Waltham), and Cape Cod Area (Great Herring, Pickerel, Mashpee, Wakeby, Santuit, Lovell's, Long, Shiverick, Sider's, Falmouth Dump, Sandwich Mill, Sandwich Fish Hatchery, and several other smaller unnamed ponds).

Despite extensive collecting during July and August in the Back Bay Fens where Tanabe reported finding infected *S. palustris,* I was unable to collect this species of snail in that immediate region. That the snail or its eggs might be washed into the region is possible because 28 young and adult *S. palustris* were collected at the anterior end of Leverett Pond and 1243 specimens were also taken in a small pool between Leverett and Ward's Pond. After 1 to 3 days isolation in 8 ounce bottles with no schistosome cercariae appearing, the snails were crushed to determine whether larval trematode infections were present. All snails were negative for schistosome cercariae. One hundred and forty of the area's most commonly observed snail, i.e., *Viviparus malleatus* (Reeve)—large imported operculate Japanese snail—were examined for trematode larvae but found negative. One *Physella heterostropha* (Say) out of 1757 collected and examined, was found to have an immature schistosome infection. It was not however of the *Schistosomatium* type but was similar to *Cercaria physellae* Talbot, 1936, and other closely related species known to be dermatitis producers. Collections of 215 *Gyraulus parvus* (Say), 200 *Amnicola limosa* (Say), 139 *Helisoma trivolvis* (Say), 123 *Galba humilis modicella* (Say), 108 *G. umbilicata* C. B. Adams, 65 *Radix auricularia* (Linnaeus)—an imported European snail and 14 *Pseudosuccinea columella* (Say) were made from various areas along the Muddy River and upon resultant examination found negative for schistosome infections.

Almost no snails were found in Hammond's Pond Area with the

exception of Hammond's Pond. Collections of 145 *Physella heterostropha,* 8 *Pseudosuccinea columella,* 9 *Planorbula jenksii* Carpenter, and 120 *Gyraulus parvus* were made and on later examination were found negative for schistosomes.

The Dedham Area was found almost barren of snails with the exception of several *Pseudosuccinea columella* in Wigwam Pond. These upon examination were found negative for schistosome infection.

Collections in all but one region of the Newton Area failed to reveal snails in significant number. An examination of 1000 *Stagnicola palustris* and 2000 *Physella heterostropha* from a small pond and stream near the crossing of Commonwealth and Walnut Avenues failed to reveal any schistosomes. Likewise, 124 *Physella heterostropha* and 65 *Helisoma trivolvis* collected below Bullough's Skating Rink Pond were negative for schistosomes.

In the Cambridge Area many *Viviparus malleatus* and *V. contectoides* Binney—operculate imported Japanese snails—*Physella heterostropha* and a few *Helisoma trivolvis* were collected from a pond on a golf course near Fresh Pond; 500 *Stagnicola palustris* from a small pool located near the Hills Crossing Station; and a few *Physella heterostropha, Viviparus malleatus,* and *Radix auricularia* from Perch Pond in the vicinity of Little River. All of these snails were found negative for schistosome cercariae.

In the Arlington and West Medford Area 500 *Physella heterostropha* were collected from Little Spy Pond, 250 *Galba humilis modicella* from a small brook draining into Little Spy Pond, 42 *Physella heterostropha* from Spy Pond, and 3 *Stagnicola palustris* from an outlet to a storm sewer on the east side of Lower Mystic Lake. All snails upon examination were found negative for schistosome infections.

In no region of the Wakefield Area is an abundant snail population found. Twenty-six *Physella heterostropha* were collected in Crystal Lake; a few *P. heterostropha* and *Helisoma trivolvis* from the Colonial Golf Club, 65 *Physella heterostropha* and 23 *Pseudosuccinea columella* from Saugus and Mill Rivers. On examination all were found negative for schistosome cercariae.

Snails were not abundant in any of the regions of Wellesley Area. No schistosome cercariae were found in 126 *Gyraulus parvus* and 38 *Physella heterostropha* collected on the Wellesley College side of Lake Waban nor in 46 *Gyraulus deflectus* (Say) collected in Jenning's Pond.

Snails were not found in sufficient numbers for collection in the Auburndale Area.

In the Cape Cod Area no schistosome cercariae were found in an examination of 251 *Gyraulus deflectus* (Say), 36 *Amnicola limosa* (Say) from Shiverick Pond; 231 *Gyraulus deflectus* (Say), 140 *Physella*

heterostropha (Say), 2 *Lyogyrus pupoideus* (Gould), 22 *Amnicola limosa* (Say), 340 *Helisoma antrosum?* (Conrad), from Sider's Pond; 145 *Pseudosuccinea columella* (Say), from Dump Pond; 500 *Physella heterostropha* from the Sandwich Fish Hatchery Pond; or in 500 *P. heterostropha* from Mill Pond, Sandwich.

DISCUSSION

At the present time there is no recognized schistosome dermatitis problem in eastern Massachusetts. Extensive collecting in various freshwater areas has failed to reveal substantial evidence that there may be such a problem in the future. For such a problem to exist in any area there must be present in fair numbers a definitive host or hosts for the schistosome cercariae, a sufficient number of intermediate hosts infected with cercariae, and then the area must be used by human beings for swimming, wading or trapping (any occupation wherein the susceptible individual's skin comes in contact with dermatitis-producing schistosome cercariae).

In many of the areas examined, the abundance of the proper snails has been markedly diminished or entirely eradicated by one or more of the following: (*a*) streams covered by oil for mosquito control or otherwise polluted by sewage, etc., (*b*) lakes and ponds prepared to serve as reservoirs (this entails building rock banks which prohibit the growth of water weeds, thereby resulting in a shortage of food for the intermediate and definitive hosts of the dermatitis producing schistosomes), (*c*) artificial, covered channels for brooks and streams, (*d*) ducks and rats in large enough numbers to markedly decrease the snail population by using the snails for food. In other areas where the proper snails are present, there has been an apparent disappearance of the definitive hosts. Where both the intermediate and definitive hosts are found present there exists a minimum of wading or swimming.

In the vicinity of the Muddy River and the Charles River Basin where Tanabe reported collecting *Stagnicola palustris* infected with schistosome cercariae in 1921 and 1922, this snail species no longer occurs. However, it is possible the snail or its eggs may be washed downstream from other areas at certain seasons of the year. No other reported intermediate host of *S. douthitti* was found in this or any other area. The only recognized definitive host found in the Muddy River area was the brown rat and Price (1931) and others have shown that the life cycle of *Schistosomatium douthitti* cannot be successfully completed in rats because the eggs fail to break through the lumen of the intestine.

Because snails in the various areas failed to reveal schistosome infections, concentrated efforts were not made to trap definitive hosts.

Marine gastropods were not collected in the present survey but it is possible that in certain marine swimming beaches or bays, they may be infected with dermatitis-producing schistosome cercariae in numbers sufficient to be a problem.

SUMMARY

1. A study has been made of five of Tanabe's preparations of *Schistosomatium pathlocopticum* and, on a basis of morphological and experimental comparison, the species demonstrated to be a synonym of *S. douthitti* (Cort, 1914), the cercariae of which are known to produce water itch or schistosome dermatitis.

2. Collections in the same areas where Tanabe (1923) found schistosome cercariae in *Stagnicola palustris* (Müller) failed to reveal the intermediate host. Examinations of 2781 *S. palustris* collected in other areas, failed to reveal infections of schistosome cercariae.

3. Extensive collecting of fresh water gastropods in 10 arbitrarily designated areas including waterways, lakes, and ponds where there is or could be swimming or wading in eastern Massachusetts, has failed to reveal a water itch problem. Examinations of 5837 *Physella heterostropha* (Say), 140 *Viviparus malleatus* (Reeve), 461 *Gyraulus parvus* (Say), 197 *Amnicola limosa* (Say), 204 *Helisoma trivolvis* (Say), 373 *Galba humilis modicella* (Say), 85 *Lymnaea auricularia* (Linnaeus), 197 *Pseudosuccinea columella* (Say), 340 *Helisoma antrosum?* (Conrad), 9 *Planorbula jenksii* Carpenter, 528 *Gyraulus deflectus* (Say), 108 *Galba umbilicata* C. B. Adams, 2 *Lyogyrus pupoideus* (Gould), from the various areas with one exception failed to reveal schistosome infections.

4. One *Physella heterostropha* (Say) collected, had an immature schistosome infection which was not of the *Schistosomatium* type but which was similar to *Cercaria physellae* Talbot, 1936, and other closely related species known to be dermatitis producers.

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PHARYNGOSTOMOIDES PROCYONIS N. G., N. SP. (STRIGEIDA) A TREMATODE FROM THE RACCOON IN NORTH CAROLINA AND TEXAS

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During the examination of twenty-three specimens of the raccoon, *Procyon lotor lotor* (L.) from North Carolina in the fall and winter of 1939–40 and 1940–41, thousands of small strigeid trematodes were found in the small intestine of fourteen specimens. These appear to represent a new genus of the family DIPLOSTOMIDAE, subfamily ALARIINAE. Mr. Allen McIntosh and Dr. E. W. Price of the U. S. Bureau of Animal Industry kindly corroborated the diagnosis. Additional material from East Texas kindly lent by Dr. A. C. Chandler necessitated revision of this description which originally was made from young specimens. The name *Pharyngostomoides* is herewith proposed for the new genus with *P. procyonis* as the type species.

Pharyngostomoides n. g.

Generic diagnosis: Family Diplostomidae: subfamily Alariinae: Body small, forebody broader and longer than hind body; pseudosuckers present; prosthetic glands usually present; holdfast organ slightly longer than broad. Oral sucker weakly developed, approximately the same size as ventral sucker. Ovary in posterior part of forebody, anterior to and smaller than testes. Mehlis' gland and vitelline reservoir between ovary and testes at junction of anterior and posterior segments. Uterus extending into mid-portion of holdfast organ. Testes approximately equal in size, usually ovoid, smooth to irregular in outline situated side by side in posterior segment. Genital cone present; genital atrium small, dorsal, subterminal. A deep dorsal furrow present in young specimens.

Type species: Pharyngostomoides procyonis n. sp.

Pharyngostomoides procyonis n. sp.

(Figs. 1–10)

Description: Body small, 0.4–1.8 mm in length; forebody spinose, scoop-shaped with lateral and posterior margins folded ventrally, longer and broader than hindbody in relaxed specimens (0.31–1.1 × 0.29–0.82 mm) often flexed dorsally; hindbody (0.17–0.89 × 0.19–0.63 mm) more or less conical. Oral sucker terminal, slightly broader than long (0.038–0.120 × 0.046–0.150 mm). Pseudosuckers well developed, incupped in young specimens, protruding ear-like in older ones; with numerous fine ducts opening into them connecting with paired masses of unicellular glands lying laterally between levels of pharynx and ventral sucker, sometimes meeting in midline dorsal to pharynx; these glands in some specimens stain deeply and constitute a striking character, but in others they appear unstained and refractile, and are sometimes barely discernible. Ventral sucker (0.058–0.120 × 0.66–0.140 mm) approximately same size as oral sucker; never raised above surface of body; between anterior margin of holdfast organ and intestinal fork; usually partially covered by holdfast organ or completely obscured by it. Holdfast organ well-developed (0.140–

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0.410×0.145–0.400 mm) slightly longer than broad; surface directed antero-ventrally with longitudinal slit-like opening in center. Prepharynx very short; pharynx (0.033–0.095×0.029–0.080 mm) usually contiguous with oral sucker; esophagus short, bifurcation anterior to ventral sucker; ceca extend to behind testes, gradually passing from dorsal side of forebody to ventral side of hindbody. Vitellaria follicular, distributed in forebody from acetabulum posteriorly to level of anterior margins of testes; slightly penetrate holdfast organ; in lateral margins extend to beyond level of acetabulum; not continuous ventrally in posterior region in older specimens (Fig. 9).

Testes variable in position, shape and size (Fig. 10); usually situated side by side in anterior part of hindbody but in 8 or 10 specimens of several hundreds examined either testis may be displaced backward; usually contiguous or close together but occasionally separated by width of testis; shape varies from spherical to long oval; size varies 0.128–0.385 mm in length and 0.089–0.240 mm in width. Right vas efferens arises from the medio-ventral surface of right testis, left vas efferens arises from the medio-anterior surface of its testis; vasa efferentia enter separately posterior surface of seminal reservoir; latter a confused mass of coils in front of ovary and between it and Mehlis' gland. Vas deferens arises from ventral side of reservoir and proceeds posteriorly between ventro-medial surfaces of opposite testes to behind latter where it becomes convoluted to form seminal vesicle; latter discharges usually through muscular ejaculatory pouch which is conspicuous in some specimens but not in others; ejaculatory duct unites with descending limb of uterus at base of genital cone. Latter often protruding beyond surface of body, thus obliterating greater part of small genital atrium. Cirrus protrusible.

Ovary small, reniform from ventral view, transversely elongated (0.035–0.120× 0.078–0.190 mm), situated dorsally in posterior part of forebody. Oviduct arises from dorso-posterior side of ovary, extending posteriad, receives common vitelline duct upon entrance into posterio-dorsal side of Mehlis' gland. Laurer's canal present; oviduct ampulla-like at its junction. Mehlis' gland and vitelline reservoir between ovary and testes often contiguous with anterior median borders of testes. Oviduct pursues sinuous course through Mehlis' gland emerging as ascending limb of uterus which continues anteriad past ventral surface of ovary to mid-portion of holdfast organ. Descending limb passes posteriad in mid-ventral plane to unite with more dorsal ejaculatory duct and empties with latter at tip of genital cone. Vitelline ducts extend posterior-mesad to ventral surface of vitelline reservoir; latter extends ventro-dorsad discharging on its dorsal side by common vitelline duct into posterior end of oviduct.

In young specimens a deep crescentic furrow present just anterior to genital cone on dorsal side; in older specimens this furrow becomes part of genital atrium. Eggs 1 to 35 in number, measuring 0.082–0.093×0.055–0.060 mm. Excretory pore terminal. Nerve strand on each side extends posteriad from region of pharynx, lateral to intestinal ceca.

Host: Procyon lotor lotor (Linnaeus).

Habitat: Small intestine.

Locality: Wake County, Raleigh, North Carolina; Martin and Pasquotank Counties, North Carolina; Angelina County, Texas.

Specimens: U. S. Nat. Mus. Helm. Coll. Nos. 44850 (Type) and 44851 (Paratypes) of R. Harkema. Also Nos. 44932 and 44933 of A. C. Chandler.

Comparatively few strigeids have been reported from animals of the family PROCYONIDAE. La Rue and Townsend (1927) described *Alaria nasuae* from *Nasua nasica* and later mentioned (1932) that an unidentified strigeid had been obtained from the raccoon. Morgan and Waller (1940) reported a case of severe parasitism of a raccoon. Several different species of parasites were found including several specimens of *Fibri-*

cola cratera (Barker and Noll, 1915). *Fibricola laruei* from the raccoon in Canada was described by Miller (1940). Chandler (1942) found *Fibricola texensis* Chandler, 1942, infecting raccoons in east Texas.

The combination of prosthetic glands, opposite testes and dorsal furrow justify the erection of a new genus. The variability of these characters possibly may militate against this viewpoint. However, only a very few specimens out of hundreds examined showed any marked shifting of the testes. The conspicuousness or obscurity of the prosthetic glands suggests the possibility of variation in the metabolic activity of these glands and their development may be influenced by the age of the flukes. These glands and the dorsal furrow show similarities with *Sphincterodiplostomum musculosus* where the pseudosuckers present the same appearance as in *Pharyngostomoides procyonis* but the glands are more diffuse. The crescentic dorsal furrow is represented by a tubular invagination with a sphincter.

Dubois (1938) lists the genera *Alaria, Cynodiplostomum, Fibricola, Pharyngostomum, Podospathalium* as members of the sub-family ALARIINAE. *Pharyngostomoides* possesses characters which separate it from these genera. It obviously is different from the aberrant *Podospathalium*. It can be separated from *Cynodiplostomum* by its smaller size, ventrally folded posterior margin of forebody, presence of a prepharynx and esophagus, prosthetic glands, distribution of vitellaria, relatively larger holdfast organ, and in the position of ovary and testes, *Fibricola* and *Alaria* both have the testes arranged tandemly; the former possesses no pseudosuckers and in *Alaria* the holdfast organ is more elongate and there are no glands. *Pharyngostomum* is the only previously described genus of ALARIINAE which has opposite testes in the hindbody. Its very large pharynx, very large piriform holdfast organ, absence of pseudosuckers and larger size validate its separation from *Pharyngostomoides*.

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Explanation of Plates

Pharyngostomoides procyonis n. g., n. sp.

Abbreviations

cvd—common vitelline duct
ejp—ejaculatory pouch
exp—excretory pore
gc—genital cone
hf—holdfast organ
int—intestinal cecum
lc—Laurer's canal
lvd—left vitelline duct
mg—Mehlis' gland
oot—oötype
ov—ovary
ovd—oviduct
rvd—right vitelline duct
sr—seminal reservoir
sv—seminal vesicle
tr—right testis
uta—ascending limb of uterus
utd—descending limb of uterus
vd—vas deferens
vel—left vas efferens
ver—right vas efferens
vit—vitelline follicle
vr—vitelline reservoir

Plate I

Fig. 1. Camera lucida drawing of whole mount of young specimen, ventral view.

Fig. 2. Camera lucida drawing of cross section through middle of holdfast organ, anterior view, ventral side uppermost.

Fig. 3. Reconstruction of male reproductive system as seen from left side in sagittal view. Left testis not shown.

Fig. 4. Partial reconstruction of cross sections at level of seminal reservoir, showing extent of Mehlis' gland, seminal reservoir, entrance and ventral limits of vasa efferentia and exit of vas deferens, descending limb of uterus and intestinal ceca. Posterior view, dorsal side uppermost.

Fig. 5. Camera lucida drawing of cross section through middle of hindbody.

Fig. 6. Reconstruction of female reproductive system as seen from left side in sagittal view. Right testis shown, right vitelline duct not shown.

Fig. 7. Reconstruction of cross sections at level of vitelline reservoir and Mehlis' gland showing oviduct, entrance of Laurer's canal and common vitelline duct, oötype, Mehlis' gland, vitelline ducts, vitelline reservoir and vitellaria. Anterior aspect, dorsal side uppermost.

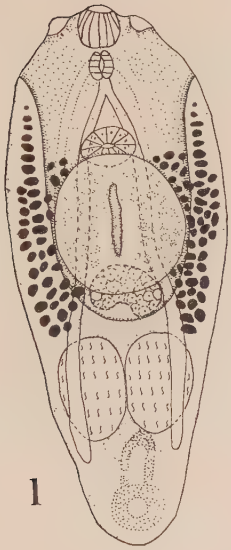
Plate II

(Drawn by A. C. Chandler)

Fig. 8. Ventral view of older specimen.

Fig. 9. Specimen showing arrangement of vitellaria and extruded cirrus.

Fig. 10. Variety of sizes and shapes observed.



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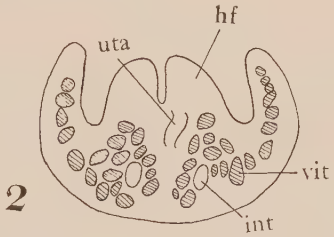

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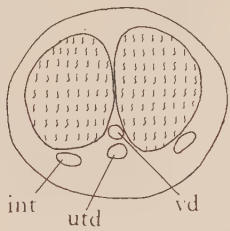

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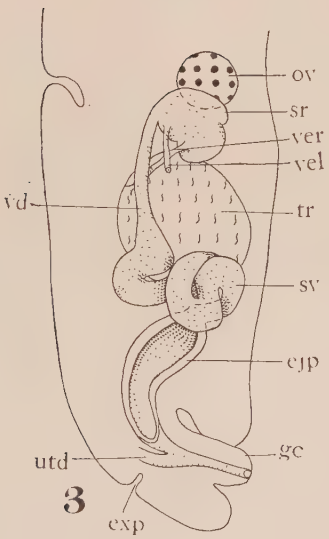

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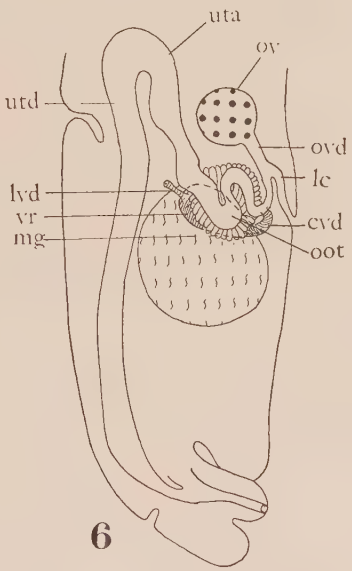

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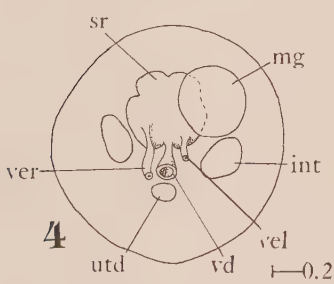

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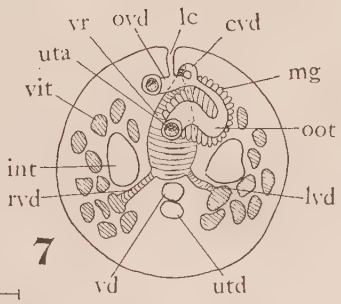

7

0.2 mm

PLATE I

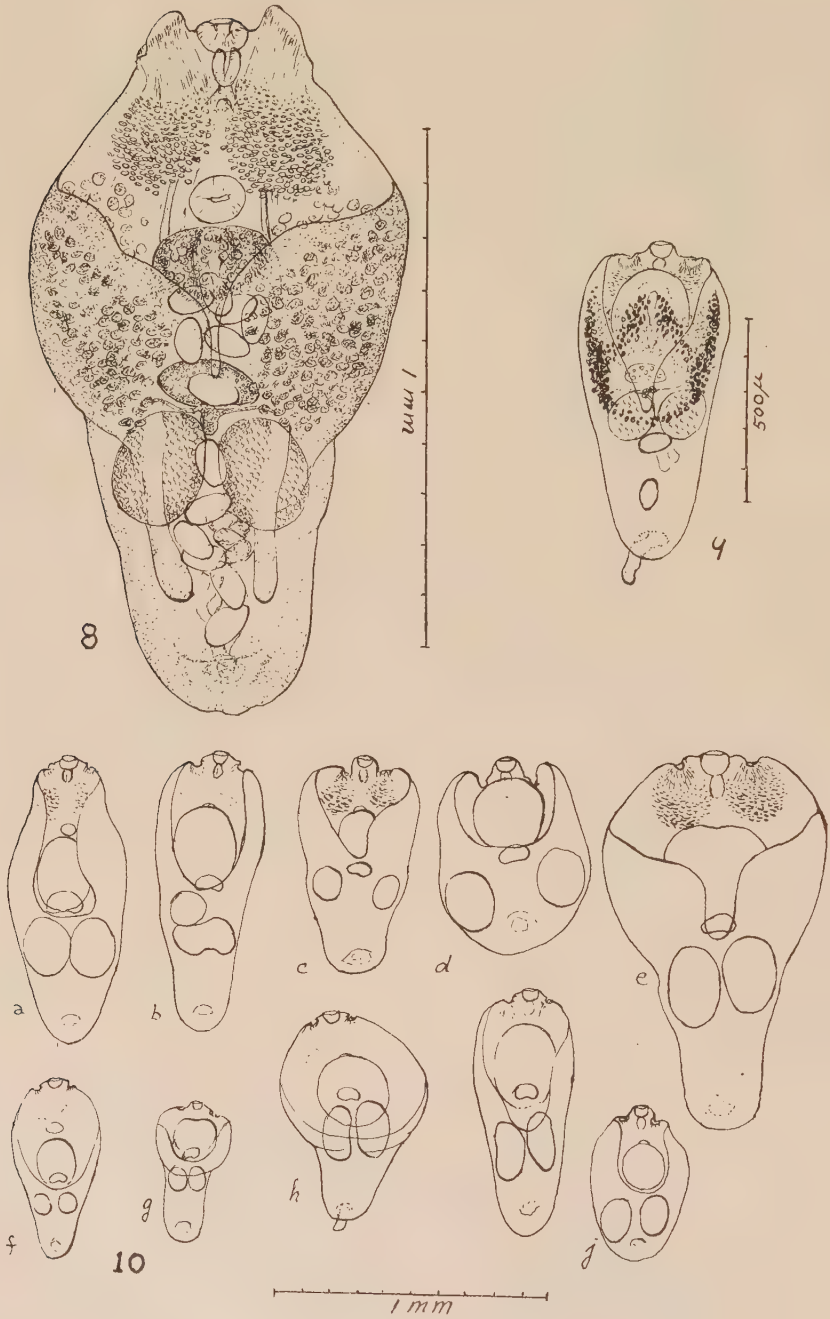

PLATE II

THE BIOLOGY AND CULTURE OF *NEOAPLECTANA CHRESIMA*, A NEW NEMATODE PARASITIC IN INSECTS[1]

R. W. Glaser, E. E. McCoy, and H. B. Girth

A few years ago a number of corn earworms (*Heliothis armigera*) near Moorestown, New Jersey, were found dead and dying from a condition which on examination proved to be due to nematode parasitism.[2] Later the present writers found the same nematode parasitic in Japanese beetle (*Popillia japonica*) larvae. From 1937 to 1940 this parasite was found among the beetle larvae in 14 places distributed among 9 counties of New Jersey and in one locality in Maryland. In all these places this nematode seemed to represent the only helminth parasite present and was apparently causing a considerable reduction in the host population. Specimens of the nematode were submitted to G. Steiner of the Bureau of Plant Industry, United States Department of Agriculture, who stated that the form represented a new species of the genus *Neoaplectana*. He will shortly publish a description of this and other new species and a key to all known forms of the genus *Neoaplectana*. Steiner furnishes the following diagnosis which is published here with his permission, the name of the species being derived from the Greek word χρήσιμα meaning useful.

Neoaplectana chresima Steiner

"*Diagnosis: Neoaplectana* resembling *N. glaseri* Steiner, 1929 (1), but the adults of both sexes different by a sharply set off, often mucronate, tail terminus, by an excretory pore opening much closer cephalad, i.e., at a latitude near the middle of the corpus of the esophagus or at about one-third of the total length of the esophagus. Spicula proximally capitate and curved outward. Arrangement of male copulatory papillae somewhat different. Larvae with four equidistant longitudinal striae on lateral fields, tail elongate-conical, terminus not set off, the phasmids located near middle.

"*Type host: Heliothis armigera* Hbn.

"*Type locality:* Moorestown, N. J., U. S. A."

Our studies showed the life cycle of *N. chresima* to be similar to that of *N. glaseri* (2). The adults of *N. chresima* are somewhat like *N. glaseri*

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[1] A study conducted cooperatively by the Rockefeller Institute for Medical Research, Princeton, N. J., the New Jersey Department of Agriculture, and the United States Department of Agriculture.

[2] On November 26, 1934, Dr. W. H. Larrimer of the Bureau of Entomology and Plant Quarantine collected three diseased pupae of the corn earworm (*Heliothis armigera* Hbn.) from the soil in an outdoor hibernation cage at Moorestown, New Jersey. These were examined by Dr. Vera K. Charles of the Bureau of Plant Industry, who found them to contain a large number of nematodes and transmitted the material to Dr. G. Steiner of that Bureau for further examination. Steiner pronounced them to be representatives of a new species of the genus *Neoaplectana*.

but smaller. As in the latter species the females are ovoviviparous, but each produces between 250 and 400 young in contradistinction to *N. glaseri,* which generally produces approximately 15 young. (Certain rare "giant" females of *N. glaseri* may produce as many as 1400 young (3).) At first it was thought that *N. chresima* females were oviparous as well as ovoviviparous because free embryonated ova were often found. After careful study, however, it was concluded that while such free embryonated ova may develop normally they were derived from mature females that ruptured in a non-isotonic solution. In an isotonic solution free ova are not obtained, and *N. chresima* females then give birth only to living young. The larval forms are smaller than the corresponding stages in *N. glaseri.* The infective stage and the adults are more sluggish. The second-stage *N. chresima* larvae ensheath more rapidly than is the case with *N. glaseri.* Dead Japanese beetle larvae parasitized by *N. chresima* rarely smell badly, and when they do the parasites are usually dead. In general, the grub cadavers assume a dirty dull yellow color in contradistinction to an ocherous brown color of those parasitized by *N. glaseri.* In the former case the larval contents become quite viscid and of a dirty yellow hue. In the latter the contents are more fluid and of a clear brown color. When Japanese beetle larvae are experimentally exposed to soil or food contaminated with *N. chresima* they die in about 4 days.

Other hosts experimentally infected with *N. chresima* were the grasshopper (*Melanoplus* sp.), the European corn borer (*Pyrausta nubilialis*), the corn earworm (*Heliothis armigera*), and catalpa sphinx caterpillars (*Ceratomia catalpa*). In one of these experimentally infected catalpa sphinx caterpillars the nema reproduced to such an extent that at death 1,140,000 second-stage larvae were counted.

It was important to know whether *N. chresima* could be cultured on artificial media as is the case with *N. glaseri* (2). Occasionally *N. chresima* would show slight development in some particular medium, but it could not be carried through a second transplant. Such cultures often smelled badly, and it was assumed that bacteria were responsible for the failures. Second-stage larvae of *N. chresima* were then subjected to a sterilizing process described in 1940 (4, 5). After the nemas were bacteria-free they grew well on pieces of fresh, sterile rabbit kidney and survived in transplants.

The method was the following. A piece of kidney weighing one or two grams is placed in the water of condensation at the bottom of an ordinary nutrient agar slant after which the surface of the tissue is inoculated with the nematodes. To prevent evaporation the cotton plug of the tube is trimmed and pushed down, and the surface covered with sealing wax. By means of a hot wire a small perforation is later made through the hardened wax. All tubes are held in a slanted position and

incubated at room temperatures, 22–28° C. The nutrient agar is not a necessary nutritional constituent. Kidney alone suffices, but under the conditions described above the slanted agar affords a surface upon which the nematodes can migrate when they are impelled to do so. Some of these sterile cultures of *N. chresima* have been maintained for about two years and are to date in their 28th transplant. Transplants may be made at intervals of one to three months.

Recently an autoclaved medium has been devised which gives excellent growth and, because it can be more cheaply prepared in larger amounts, has replaced the older fresh rabbit tissue method. This medium is a semisolid gel at pH 7.0, in the proportion of 20 gm ground beef kidney or liver, 100 ml water, 0.5 gm sodium chloride, and 0.5 gm agar. This is autoclaved and may be stored for two weeks prior to use. It may be used for *N. glaseri* as well as for *N. chresima,* although it is not the substrate which is usually employed for the former species. Since both species are aerobic they develop on and near the surface of the semisolid gel. This gel offers enough resistance so that the nemas do not perish by sinking and further obtain enough foothold, so to speak, to effect their molts. Every two days the flasks containing the cultures are gently shaken to bring fresh nutrients from the lower layers to the surface. Under these conditions the cultures ordinarily reach stability in about 15 to 30 days, depending upon the amount of medium, the number of nemas initially used as inoculum, and fluctuations in the daily temperatures. When the cultures have reached stability no further increase in numbers is obtained on longer incubation and the dominant form is the infective larva.

A comparative count on the above medium for *N. glaseri* and *N. chresima* follows. Fifty ml of medium were used throughout, and the flasks were initially inoculated with approximately 10,000 infective forms of *N. glaseri* or *N. chresima* respectively. The final counts (recorded below as the average of 5 separate samples) were made in 23 days, at which time these cultures had reached stability. The cultures were free of all microörganisms, as comprehensive sterility tests showed.

Nutrient in medium	Final count for *N. glaseri*	Final count for *N. chresima*
Beef liver	538,000	522,000
Beef kidney	428,000	386,000
Beef muscle	18,000	6,000

The above test shows that the two species grew well on both liver and kidney but not at all on beef muscle. The counts on the last nutrient, within the experimental error, indicate no increase above the number initially introduced.

In 1940 (5) the importance of sterility was emphasized in rearing *N.*

glaseri to obtain the maximum yield. As regards *N. chresima,* no growth is ordinarily obtained when contaminants are present. However, in the presence of one bacterium a number of strains of *N. chresima* have developed and have consistently yielded cultures on transplantation. This contaminant is a nonputrefactive, nonsporulating, gram negative, motile bacillus originally derived from the soil. Cultures of *N. chresima* associated with this seemingly innocuous bacillus are not so luxuriant as are the sterile cultures. The data below illustrate this point. The experiments were performed as previously described except that a smaller amount of medium and a smaller nema inoculum were used.

Final counts for *N. chresima* cultures
(Nutrient in medium is beef liver.)

Strain No.	Sterile	Contaminated with one bacterial species
1	266,000	199,000
2	280,000	14,000
3	300,000	86,000

The sterile cultures of *N. chresima* have now been carried by transplantation for two years, and those contaminated with the one bacillus for one and one-half years. For future physiological studies, an attempt is now being made to adapt *N. chresima* to liquid nutrients such as the nutrient solution for *N. glaseri* described in 1940 (5).

No field tests, to appraise the economic possibilities of *N. chresima,* have as yet been attempted.

SUMMARY

A new nematode, preliminarily described by Steiner as a new species, *Neoaplectana chresima,* was found naturally parasitic in two species of insect hosts, and five additional species of insects were found to be susceptible to experimental infection. Certain biological differences between *N. chresima* and *N. glaseri* are briefly outlined. The sterile culture of *N. chresima* is described and the deleterious effects of contaminants emphasized.

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SOME MOSQUITO HOSTS TO AVIAN PLASMODIA WITH SPECIAL REFERENCE TO *PLASMODIUM GALLINACEUM*[1]

PAUL F. RUSSELL AND BADRI NATH MOHAN

Brumpt (1935) described *Plasmodium gallinaceum* and later (1936 a, b) reported experimental transmission to fowls and certain other birds by *Aëdes aegypti* and *Aëdes albopictus* (*"albopicta"*). No development of the parasite occurred in *Culex fatigans*. Roubaud et al (1939) reported complete development of this plasmodium in *Aëdes geniculatus*, a tree-hole breeder of France.

Huff (1932) reviewed the literature in reference to mosquitoes found susceptible to avian malaria, irrespective of *Plasmodium* species (excluding, of course, *P. gallinaceum*) and compiled the following list:

Culex (*Culex*) *quinquefasciatus* Say
Culex (*Culex*) *pipiens* Linn.
Culex (*Culex*) *hortensis* Ficalbi
Culex (*Culex*) *territans* Walker
Culex (*Culex*) *salinarius* Coq.
Culex (*Culex*) *tarsalis* Coq.
Aëdes (*Stegomyia*) *aegypti* Linn.
Aëdes (*Ochlerotatus*) *mariae* Ed. Serg.
Aëdes (*Ochlerotatus*) *communis* De Geer
Aëdes (*Finlaya*) *triseriatus* Say
Culicella longeareolata Macq.
Anopheles (*Myzomyia*) *subpictus* Grassi

Nono (1932) added *Lutzia fuscana* Wied. [*Culex* (*Lutzia*) *fuscananus* Wied.] to this list. Reichenow (1932) demonstrated the susceptibility of *Theobaldia annulata*. Herman (1938) added *Aëdes sollicitans* and *Theobaldia melaneura*.

We have found the following species to be suitable hosts for *P. praecox*, of local sparrows, in our laboratory:

Culex (*Culex*) *bitaeniorhynchus* Giles, 1901
Culex (*Culex*) *gelidus* Theo., 1901
Culex (*Culex*) *whitmorei* (Giles) 1904
Culex (*Culex*) *theileri* Theo., 1903

The chief purpose of this note is to record the development of *P. gallinaceum* in several other species of mosquitoes found in the Nilgiris area of Madras Presidency, India. The strain of *P. gallinaceum* used origi-

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[1] These studies were made under the auspices and with the support of the International Health Division of The Rockefeller Foundation co-operating with the Pasteur Institute of Southern India at Coonoor, Nilgiris, and with the Madras Provincial Health Department.

nated in Ceylon and was sent to the authors by courtesy of Lt.-Col. H. E. Shortt, I.M.S., Director of the King Institute of Preventive Medicine, Guindy, Madras. The infections in these mosquitoes were developed in connection with a study of sporozoite agglutination reported elsewhere (Mulligan et al, 1940; Russell et al, 1941).

Complete exogenous development of *P. gallinaceum* has been observed by us in the following species fed on infected domestic fowls:[2]

Armigeres (*Armigeres*) *obturbans* (Walk.) 1860.
Breeds mostly in cut bananas, but also in tree holes, cut bamboos, and domestic water, often very foul.
Armigeres (*Armigeres*) *kuchingensis* Edwards, 1915.
Breeds in cut bananas and bamboos.
Armigeres (*Armigeres*) *aureolineatus* Leicester, 1908.
Breeds in cocoanut shells.
Aëdes (*Stegomyia*) *aegypti* (Linn.) 1762.
Breeds in domestic water in artificial containers.
Aëdes (*Stegomyia*) *albopictus* (Skuse) 1894.
Breeds in tree holes, cut bamboos and bananas, cocoanut shells, and rarely in domestic water.
Aëdes (*Stegomyia*) *unilineatus* (Theobald) 1906.
Breeds in tree holes.
Aëdes (*Stegomyia*) *pseudalbopictus* Borel, 1928.
Breeds in cut bamboos, cocoanut shells.
Aëdes (*Stegomyia*) *vittatus* (Bigot) 1861.
Breeds in tree holes, rock pools, cocoanut shells, and domestic water.
Aëdes (*Stegomyia*) *scutellaris* (Walker) 1859.
Breeds in tree holes, cocoanut shells, and domestic water.
Aëdes (*Finlaya*) *pseudotaeniatus* (Giles) 1901.
Breeds in tree holes, rock pools, domestic water.
Aëdes (*Finlaya*) *chrysolineatus* (Theobald) 1907.
Breeds in tree holes, cut bamboos, rock pools, and domestic water.
Aëdes (*Finlaya*) *pallirostris* Edwards, 1922.
Breeds in rock pools and tree holes.
(Specific names from Barraud, 1934).

We have failed in repeated attempts to obtain development of *P. gallinaceum* in the following species:

Culex (*Culex*) *fatigans* Wiedemann, 1828.
Culex (*Culex*) *mimeticus* Noe, 1899.
Culex (*Culex*) *bitaeniorhynchus* Giles, 1901.
Anopheles stephensi Liston, 1901.
Anopheles jeyporiensis James, 1902.

All mosquitoes after feeding on infected fowls were kept in an insectary where temperature was maintained between 70° and 85°F and relative humidity between 75 and 85 per cent. Sporozoites in all susceptible species were seen after incubation periods of from 9 to 12 days.

Four species, *Armigeres obturbans, Armigeres kuchingensis, Aëdes albopictus,* and *Aëdes aegypti,* were found most suitable for the develop-

[2] Our diagnoses of species have been confirmed by courtesy of Dr. I. M. Puri, Entomologist, Malaria Institute of India, Delhi.

ment of very heavy sporozoite infection of salivary glands. The *Armigeres* species would feed on fowls at any time during daylight but especially well in the afternoon. The *Aëdes* species fed most avidly in the morning but would also feed at other times, even at night. Insectary colonies of *obturbans* and *albopictus* have been maintained without difficulty.

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A SIMPLE APPARATUS FOR DETERMINING THE VIABILITY OF EMBRYONATED HELMINTH OVA

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One of the major problems confronting workers in making studies on the effects of such agencies as chemicals, heat, cold, ultra-violet radiation and ultra-short radio waves (Hertzian waves) on helminth ova has been the application of a suitable test for viability. It is easy to subject unembryonated eggs to chemical and physical variants and to use subsequent development as indicative of life. If the ova are embryonated, on the other hand, the problem is more complicated, since the only suitable test is motility. Without some external stimulation the encapsulated larvae may remain quiescent for long periods causing the observer to spend many hours at the microscope before an accurate pronouncement can be made. This is especially true in longevity experiments during which the natural activity of the embryo decreases with the increase in the time of incubation. The method described below activated eggs of *Heterakis gallinae* and *Ascaridia galli* from cultures nearly two years old, and allowed making a rapid determination of viability on a large series.

Before the development of the apparatus described below, various unsatisfactory methods were used in attempting to stimulate activity on the part of embryonated eggs; including electric shock, exposure to strong light rays, and placing the eggs in an electric oven before preparing them on slides for observation. The results were so variable as to render these methods unreliable. A modification of the Lamson-Brown (1938) technique was then tried, with slide preparations being placed over a beaker containing steaming-hot water. This gave fair results, but the condensation of water on the slide required wiping it; often the ova were killed by too intense heat, and very often the larvae, if stimulated to activity by the process, apparently returned to quiescence before observation was made.

The method which gave the basic idea for the apparatus described below produced excellent results, activating larvae to observable motion even in the oldest cultures in the laboratory. A piece of copper wire about six inches long was coiled at one end, the coil being somewhat less in diameter than the aperture in the stage of the microscope and open in the center to admit the light from the condenser. The wire was then bent at an obtuse angle about one inch from the coil, and the coil angled

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in the opposite direction to the same degree. The instrument thus formed had an open sigmoid shape, the wire handle and heating coil being parallel. Slide preparations were placed on the microscope, a suitable ovum selected microscopically, and the condenser lowered about one inch, the light being adjusted to give good visibility. The coil was then heated over a burner until red and applied through the stage aperture to the slide.

Even though heating the ovum from below with a hot wire gave the desired results there were some obvious difficulties to the method. Often

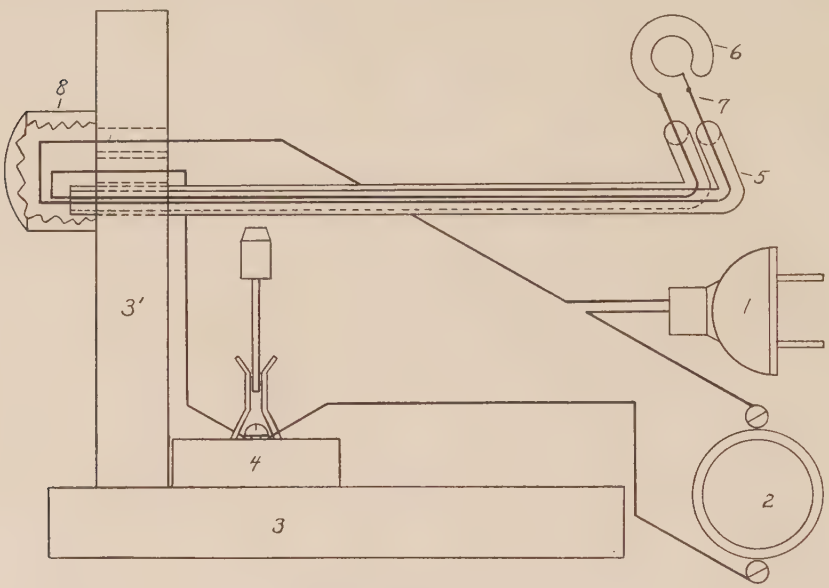

FIG. A. Apparatus to determine the viability of helminth eggs. Side view. (1) Electric cord plug; (2) Socket for 150-Watt bulb; (3, 3′) Wooden support: (4) Knife-blade switch; (5) Glass tube supports of the heating element; (6) High resistance heating wire; (7) Copper wire leads to heating element; (8) Celluloid cap over the naked wire junctions.

the hot wire came into contact with the stage, and occasionally the glass parts of the microscope were touched. Sometimes the slide dried rapidly preventing sufficient observation. To obviate these difficulties a simple electric heating element was devised (Figs. A and B) which gives excellent results, viable embryos being stimulated to activity in a very short time. The apparatus is cheap in construction, and saves many hours of tedious observation when a large series of cultures is to be tested.

The equipment needed is about 5 feet of ordinary electric light cord attached to a socket plug; a 150-Watt standard bulb and a socket; 3 feet of #18 copper wire; a few inches of #30 high resistance wire for the heating element; a standard knife-blade switch; two pieces of thick-

walled, high melting-point glass tubing with an inside diameter of 1 mm; two blocks of wood to serve as a support, and the necessary screws to attach the socket and switch to their respective supports.

The socket with the 150-Watt light bulb and the switch are connected in series to the light cord. The two glass rods are cut into lengths of $10\frac{1}{2}$ inches and bent at right angles about $3\frac{1}{2}$ inches from the end. Careful, uniform heating of the tubes must be applied so as not to reduce the inside diameter in the bent area. Two lengths of copper

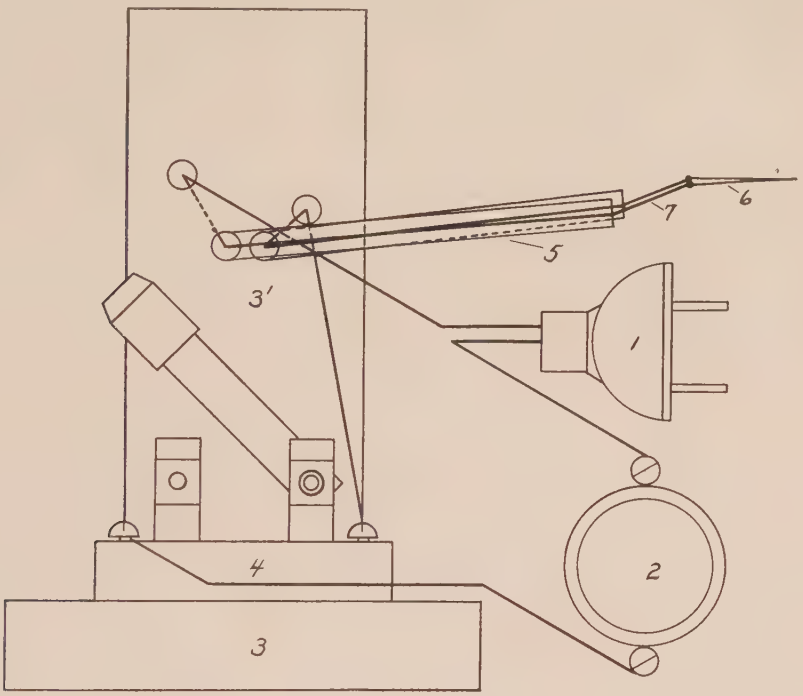

FIG. B. Front view of viability testing apparatus showing the heating element (6) in position. The parts of the apparatus are numbered as in Fig. A. The wiring is indicated by heavy lines.

wire are cut, each 12 inches in length, and the insulation removed. These are threaded through the glass tubes from the long end. Since the wire used nearly fills the glass conduit it must be worked through slowly, with constant twisting, until about $\frac{1}{2}$ inch projects from the short end from the angle. The high resistance wire (about 2 inches long) is then soldered into place on the protruding ends of the copper wire using acid core solder, the two copper wires thus being joined, and insulated in glass. The glass rods are inserted at the desired height by the long end from the angle into the upright of the stand formed from the two wooden blocks. By careful choice of bits for boring the holes a snug fit for the glass tubes results so that no additional support is required. The free

wires projecting from the back of the upright are soldered into the circuit and the junctions insulated with tape. In the case of the instrument figured the celluloid covering of the cork of a chemical bottle was taped to the support over the naked wires. When the circuit has thus been completed the high-resistant wire is moulded into a coil of the desired diameter, care being taken so that the wire loops in the heating element thus formed do not touch. The copper poles to which the coil is attached are bent so that the coil lies parallel to the stage in the desired position in the stage aperture, the wooden support being located at the side of the microscope. By simply closing the circuit with the key, the heating coil becomes radiant and the observer can watch the reactions of the embryo in the egg even under the high powers of the microscope. At the first signs of motility, observable in our tests in less than a half minute, the switch is opened. The amount of heat supplied can be governed by the size of the bulb in the circuit. The larger the bulb, the greater the amount of heat radiation. A 150-Watt bulb will give sufficient heat without causing the heating unit to become red, thus reducing the period of its effectiveness. Fig. A shows a side view of the apparatus, and Fig. B the front view.

HELMINTHS OF TREE SQUIRRELS IN SOUTHEAST TEXAS

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In cooperation with Mr. Rollin H. Baker, Field Biologist of the Texas State Game, Fish and Oyster Commission, engaged in ecological studies of native fur animals in southeast Texas, 12 fox squirrels, *Sciurus niger rufiventer,* and 4 gray squirrels, *Sciurus carolinensis carolinensis,* from Angelina, Hardin and Trinity Counties in southeast Texas were examined for helminths. One additional specimen from the Rice Institute campus was examined, and is included in the percentages of parasitic infections mentioned below. No significant differences were noted in the parasitic fauna of squirrels from the several localities represented.

Members of the genus *Sciurus,* as far as can be judged from the literature, are not often heavily parasitized, and the parasites reported vary widely from place to place. In Hall's (1916) monograph on nematodes of rodents, *Trichostrongylus delicatus* was the only nematode reported from squirrels in North America, and it was described as a new species. *Heligmodendrium hassalli* (Price, 1925) is the only helminth so far found to be prevalent in tree squirrels in this country. First reported from Maryland, it was found by Harkema (1936) in over 90% of gray squirrels (*Sciurus carolinensis*) in North Carolina and is here reported from every specimen examined of both gray and fox squirrels from East Texas. It is presumably widely distributed in the southern states. Several kinds of larval tapeworms have been reported from tree squirrels both in this country and in Europe, but *Raillietina* (*Raillietina*) *bakeri* herein described is, so far as I am aware, the only adult tapeworm yet described from *Sciurus* in North America; in Europe *Catenotaenia dendritica,* a member of the family Dilepididae, is a familiar parasite of

Table 1.—*Helminths of tree squirrels in southeast Texas*

	Sciurus niger rufiventer 13 examined		*Sciurus carolinensis carolinensis* 4 examined	
	No.	%	No.	%
Cestodes				
Raillietina (R.) bakeri ...	8	61	0*	0
Cysticercus tenuicollis	1	8	0	0
Nematodes				
Strongyloides robustus ...	11	85	2*	50
Heligmodendrium hassalli .	13	100	4	100
Microfilaria alpha	1	8	0	0
Microfilaria beta	1	8	0	0

* Two of the gray squirrels and two of the fox squirrels examined were young. All four of these animals were free of all helminths except *Heligmodendrium hassalli.*

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squirrels, and a *Hymenolepis* (*H. myoxi sciurina*) has been reported from these animals in Europe and Asia. No trematodes, so far as I am aware, have hitherto been found in tree squirrels, nor were any found in the present investigation.

More detailed data on the helminths found, with descriptions of the new species, follow.

Raillietina (*Raillietina*) *bakeri* n. sp.
(Figs. 1–3)

Delicate worms reaching length of about 60 mm with maximum width of about 1.2 mm. Scolex 250 to 375 μ in diameter, broadest at middle, and without sharp demarcation from neck. Rostellum surrounded by double crown of about 66 hooks appearing as single row, alternate hooks being set only about 2 μ out of line; hooks about 20 to 22 μ long and about 13 μ across the hammer-shaped head; diameter

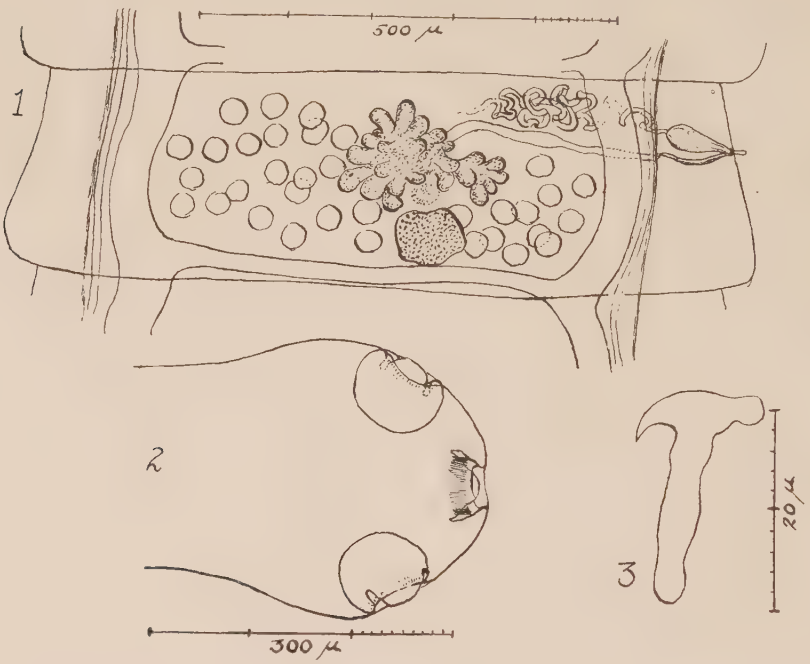

Fig. 1. *Raillietina* (*R.*) *bakeri*, n. sp. Mature segment.
Fig. 2. Same, scolex.
Fig. 3. Same, rostellar hook, greatly enlarged.

across top of crown of hooks 65 to 70 μ. Suckers 80 to 100 μ in diameter with plug of tissue filling most of cup, and provided with very minute spines, 4 to 5 μ long, in at least two rows; most specimens, however, devoid of spines after preparation. Beginning of segmentation about 3 to 5 mm behind anterior end. Neck variable in diameter, according to state of contraction; in well-relaxed specimens usually about 220 to 250 μ, but only 130 μ in one specimen.

Mature segments 680 to 850 μ broad and 175 to 300 μ long; segments showing beginning of uterus usually somewhat narrower but longer, about 550 to 700 μ broad by 400 μ long; segments with developing egg capsules about 800 μ to 1 mm broad and 400 to 450 μ long; undetached segments with egg balls about 1.1 to 1.2 mm

broad, and nearly square. Detached ripe segments usually single or in groups of 2 or 3, from 1.75 to 2.25 mm long and 800 μ to 1 mm broad.

Ventral excretory vessels very broad and conspicuous, up to 60 μ in diameter; transverse vessels slightly narrower; dorsal vessels inconspicuous, seldom seen in older segments. Longitudinal nerve close to dorsal vessel. Genital pores unilateral, situated in anterior third of segment. Cirrus pouch 90 to 95 μ long and about 35 μ wide, not reaching excretory vessels; cirrus unarmed. Vas deferens very much convoluted, occupying a considerable space in anterior poral corner of the segment. Testes 30 to 40 in number, about 18 to 24 of them occupying space on aporal side of segment between female glands and excretory vessel, and 11 to 16 of them on poral side behind genital ducts; in young segments one or two testes may be immediately behind female genital glands, thus connecting the two fields; in older specimens one or two may be dorsal to genital glands. Testes about 28 to 38 μ in diameter, often oval or slightly irregular in shape. Vagina posterior to vas deferens, with muscular deep-staining wall as far as excretory vessels, then becoming widened with thin walls, and serving as seminal receptacle. Ovary situated slightly towards poral side of segment, tending towards division into two main lobes, aporal one the larger, but made up of numerous small finger-like lobes. Yolk gland about 90 to 95 μ in diameter, behind ovary, slightly towards poral side of segment. Segments with developing egg balls detach and finish their development free from strobila, resembling oval seeds. Egg balls remain between excretory vessels, but in fully ripe segments walls of segment close down on egg balls like a capsule, and no traces of excretory vessels or genital ducts can be seen. Egg balls about 80 to 90 in number, round or oval, from 140 to 195 μ in diameter, and containing from 6 to 9, occasionally 10, ova.

Host: Sciurus niger rufiventer.

Habitat: Small intestine.

Locality: Southeast Texas.

Type specimen: U. S. Nat. Mus. Helm. Coll. No. 44916. *Paratypes* (4 slides) No. 44917.

Only three species of *Raillietina* have hitherto been recorded from North American rodents, namely *R.* (*Fuhrmanetta*) *salmoni* from rabbits, *R.* (*Paroniella*) *retractilis* from rabbits, and an undescribed species from a gopher. This is the first species of the subgenus *Raillietina* from North American rodents, and the first adult tapeworm of any kind to be reported from North American tree squirrels. In the small number of testes, small cirrus pouch, and small number of uterine capsules *R. bakeri* more nearly resembles *R. loechesalavezi* Dollfus 1939–1940, described from a specimen from an 18-year-old child in Cuba, than any other form described from mammals. From this it differs, however, in its smaller size, the smaller size of the cirrus pouch, the bilobed uterus, and the failure of the testes to extend beyond the excretory vessels.

Cysticercus tenuicollis

A single small *Taenia* cyst was found attached to the liver of a fox squirrel. The head and neck, evaginated from the cyst but with the scolex invaginated into the neck, measured about 1.85 mm in length, while the cyst measured about 2.2 mm in length. The number, size and shape of the hooks, as well as the general form, indicate that this is a small, probably young, *Cysticercus tenuicollis,* the larva of *Taenia hydatigena.*

This species has previously been reported from squirrels. *Cysticercus fasciolaris* and an unknown cysticercoid with a scolex provided with a double crown of small hooks about 15 μ long (see Schwartz, 1928) have also been reported from tree squirrels in North America.

Strongyloides robustus n. sp.

Parasitic female. Length of egg-bearing worms varying from 4.5 to 6.8 mm, but 6 mm or more in 10 of 13 specimens measured and falling under 5.5 mm in only one case; average length 6.1 mm. Maximum diameter 60 to 75 μ except the 4.5 mm worm, which measured 40 μ. Diameter at base of esophagus 50 to 65 μ except in two worms, in which it was 36 and 40 μ respectively. Cuticle very finely striated. Esophagus 860 μ to 1.26 mm long, averaging 1.14 mm, and varying from 17.7 to 19% of the length of the body. Anus 70 to 80 μ from caudal tip except in the 4.5 mm worm, in which it is 60 μ distant. Vulva 2 to 2.88 mm from posterior end (except 1.6 mm in the 4.5 mm worm), the post-vulvar length representing 35% to 43% of entire body length. Anterior uterus reflexed 325 to 440 μ behind posterior end of esophagus, and posterior uterus 100 to 260 μ anterior to anus. Reflexed loops usually twisted, but to various degrees. Number of eggs in the combined uteri from 3 to 20, averaging 9. Eggs 55 to 60 μ by 27 to 30 μ.

Hosts: Sciurus niger rufiventer (type) and *S. carolinensis carolinensis.*

Habitat: Small intestine.

Locality: Southeast Texas.

Type specimen: U. S. Nat. Mus. Helm. Coll., No. 44911.

This is the largest species of *Strongyloides* thus far described except *S. westeri* of horses. It resembles *S. papillosus* in most respects but is larger, the majority exceeding the maximum size reported for *papillosus.* The esophagus is both relatively and absolutely longer, its length in every instance exceeding the maximum length in *papillosus,* and averaging about half again as long as the average for that species. The minimum diameter of the body is equal to the maximum for *papillosus* in all but a single undersized specimen of 13 measured. The vulva in *papillosus* is from 1.6 to 2 mm from the posterior end whereas in *robustus* it is from 2 to 2.88 mm distant, again excepting one undersized specimen. The ovaries are less twisted than in *papillosus.* The egg measurements of *papillosus* are given as 40 to 60 μ by 20 to 25 μ, whereas those of *robustus* are 55–60 μ by 27–30 μ. It differs from *S. agoutii* Griffiths, 1940, in greater size, relatively greater thickness, relatively shorter esophagus, greater distance of vulva from posterior end, larger eggs (46–58 μ by 23–29 μ in *agoutii*). It differs markedly from *ratti* (1) in size; (2) in having an esophagus which, though always longer, is shorter relative to the body length; and (3) in size and shape of the eggs.

This worm was found in moderate numbers in all of the adult fox and gray squirrels examined, but was absent in two specimens of each species which were not full grown.

Heligmodendrium hassalli

This heligmosome was first described by Price (1929) under the name *Heligmostrongylus hassalli* from a gray squirrel, *Sciurus caro-*

linensis, in Maryland, and was subsequently reported by Harkema (1936) from over 90% of specimens of the same host in North Carolina. Dikmans (1931) transferred this species to the genus *Longistriata,* but Travassos (1937) placed it in a new genus, *Heligmodendrium,* on the basis of the interrupted cuticular striations.

Morphological characters and measurements of the Texas worms correspond closely with Price's description of the worms obtained in Maryland, except that the Texas females reach a length of 9 to 12 mm as compared to 8.4 to 8.6 given by Price. In Price's paper the width of the spicules at the base is given as 16 μ, whereas according to my measurements they are only 5 to 6 μ. Examination of Maryland specimens kindly supplied by Dr. Price showed that "16 μ" was evidently a typographical error for 6 μ.

H. hassalli was found, usually in moderate numbers, in every fox squirrel and gray squirrel examined from East Texas, and was the only helminth present in young, half-grown specimens.

Microfilaria alpha n. sp.

Slender unsheathed microfilariae 207 to 237 μ in length and about 3.5 μ in diameter. Head end bluntly rounded, tail drawn out into a fine point. Specimens dried in blood smears lie nearly straight, with only one or two bends in body. Anterior end free of nuclei for distance of about 3 to 4 μ, but nuclei extend almost to tip of tail. Nerve ring 46 to 54 μ (average 50 μ) from anterior end; excretory pore 12 to 17 μ beyond nerve ring. Anal cell 36 to 40 μ from posterior end (average 38 μ). Approximate percentages of body length as follows: nerve ring, 23%, excretory pore, 29%, last genital cell, 70%, anal cell, 82%.

About 150 to 170 μ from anterior end, and 25 to 30 μ anterior to anal cell, a large and conspicuous break in nuclear column representing third "G" cell. About 8 to 12 μ in front of this a somewhat less conspicuous break, probably representing second "G" cell; first "G" cell represented by inconspicuous break, not always seen, about 5 to 10 μ farther anterior. Abundant in blood smears of one specimen of *Sciurus niger rufiventer* from Hardin County.

Host: Sciurus niger rufiventer.

Habitat: Blood.

Locality: Hardin County, Texas.

Type specimens: U. S. Nat. Mus. Helm. Coll., No. 44912. *Paratype,* No. 44913.

Microfilaria beta n. sp.

Slender unsheathed microfilariae 125 to 150 μ in length with maximum diameter of about 3.5 μ. Specimens dried in blood smears lie curled in rather kinky manner. Head end bluntly rounded and devoid of nuclei for about 4 to 5 μ. Nerve ring situated about 33 to 40 μ from anterior end (average 36.5 μ). Excretory pore not recognizable. Inconspicuous break in column of nuclei about 75 to 80 μ from anterior end, followed by two others about 7 or 8 μ apart, of which last is largest and most conspicuous. Anal cell 35 to 45 μ from tip of tail. Percentage distances from anterior end average about as follows: nerve ring, 28%, last (largest) genital cell, 69%, anal cell, 85%. This microfilaria was found in very small numbers in blood smears from one specimen of *Sciurus niger rufiventer* from Houston, Texas.

Host: Sciurus niger rufiventer.

Habitat: Blood.

Locality: Houston, Texas.

Type specimens: U. S. Nat. Mus. Helm. Coll., No. 44914. *Paratype,* No. 44915.

Very careful search of the carcasses of these two squirrels showing microfilariae in their blood failed to reveal the adults. The body cavities, mesenteries, subcutaneous tissue, inter-muscular fasciae, sinuses, eye sockets and other parts, after prolonged examination, were digested with artificial gastric juice, but without revealing any trace of the adult worms. Hall (1916) reports two species of *Filaria* as having been reported from tree squirrels: *F. pistillaris* Molin, 1858 from Brazil, and *F. linstowi* Hall, 1916 from Siam. Since then *Litomosoides carinii* has also been reported from squirrels, by Travassos in Brazil. The first two species, found in subcutaneous tissue, are very inadequately described and their microfilariae are unknown; *Litomosoides carinii,* abundant in Texas cotton rats (*Sigmodon hispidus*) inhabits the pleural cavities and has sheathed microfilariae. It is probable that both the microfilariae described above represent hitherto undescribed species.

SUMMARY

Thirteen fox squirrels and four gray squirrels from East Texas harbored the following helminths: *Raillietina* (*R.*) *bakeri,* n. sp., in a majority of the adult animals; *Cysticercus tenuicollis,* one specimen; *Strongyloides robustus,* n. sp., in all adult animals; *Heligmodendrium hassalli,* in all animals, even those not full grown; and two kinds of microfilariae, each occurring once.

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HYMENOLEPIS MASTIGOPRAEDITA, A NEW CESTODE FROM A PINTAIL DUCK*

SEIGUL J. POLK

A pintail duck, *Anas acuta tzitzihoa* Vieillot, collected near Stillwater, Oklahoma, during the fall of 1940, was found to be heavily infected with tapeworms among which were several specimens of an apparently new species herein described as *Hymenolepis mastigopraedita*. The worms were fixed in hot Bouin's fluid and stained in borax carmine. All studies and measurements were made on 11 mounted specimens, 7 of which remain intact and are herewith designated as cotypes.

Hymenolepis mastigopraedita n. sp.
(Figs. 1–4)

Description: Strobila 57–68 mm long, 1.5 mm in maximum width, and about 250 μ thick posteriorly. Longest example with 183 proglottids. No scoleces were found—in most of the specimens, however, the strobila terminates anteriorly in a knot-like body comprising several immature proglottids and regarded as a pseudoscolex formed apparently by a back-and-forth folding of the front end of the chain followed by adhesion of juxtaposed surfaces, the segments involved being individually recognizable and not noticeably peculiar in form. Youngest available proglottids, 140 μ long, 54 μ wide anteriorly, and 98 μ wide posteriorly. Mature segments somewhat trapezoidal being about 350 μ long, 700 μ wide anteriorly, and 840 μ wide posteriorly. Most fully developed segments, only partially gravid, about 450 μ long and 1.5 mm in maximum width. First-formed proglottid, still present on most of the worms, of diminutive size, rounded posteriorly, and sterile; contains common pore of ventral excretory canals.

Genital apertures unilateral, dextromarginal, and slightly preequatorial. Genital atria bell-shaped, narrow, transversely elongate, and provided each with a prominent, spheroidal, thick-walled, finely spinous sacculus accessorius about 168 μ in diameter in mature segments and located dorsosubanteriad to distal portion of cirrus pouch. Gonaducts dorsal to poral longitudinal urinary canals. Two pairs of longitudinal excretory vessels—ventral ones broad, slightly undulant, and united posteriorly in first-formed segment; dorsal ones very narrow and more sinuous; no transverse commissures observed.

All measurements recorded below for reproductive structures represent averages based upon findings in mature segments.

Ovary comprises two very unequal, irregularly lobulated, and widely separated wings connected by an elongate, narrow, transverse isthmus; greatest diameter of wings—poral 182 μ, aporal 350 μ. Vitellarium 112 μ in diameter, slightly lobed, centrally located between and behind wings of ovary. Female genital pore immediately ventral to male pore. Distal part of vagina an exceedingly tenuous tube, hair-like in appearance; coursing inward from the atrium it makes a single left-hand spiral turn around distal portion of cirrus pouch, passing successively along posterior, dorsal, and anterior surfaces of the latter—the curvature of the spiral sometimes smooth and direct, sometimes complicated by minute sinuosities. Seminal receptacle smoothly

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ovoidal, transversely elongate; some 200 μ long in mature proglottids it becomes very much larger in older ones; submedially located in anterior part of segment ventral to cirrus pouch. Mehlis' gland, oviduct, vitelline duct, and portion of vagina proximal to receptacle—all not observed. In older proglottids, the uterus, partially gravid, nearly fills the segment; it is transversely elongate, irregularly provided with outpouchings, and extends laterally above and beyond excretory canals. No fully gravid segments nor fully developed oncospheres were encountered.

Testes three in number, 308–320 μ in diameter, one poral and two aporal; in rather uniform arrangement—two of them being posteriorly located on opposite sides of the median line with the third anterosinistrad from them, all on a level dorsal to the excretory canals and ovary. Vasa efferentia not observed. Cirrus pouch, very prominent and nearly straight in mature segments, 770 × 90 μ, extends obliquely across body often to protrude into next preceding segment and often to underlap the aporal excretory canals; variously curved and coiled in younger stages. External seminal vesicle prominent, somewhat variable in position but mostly dorsal to cirrus pouch, about 420 × 112 μ. Internal seminal vesicle, 80 μ in diameter, largely fills aporal half of cirrus pouch. The two vesicles connected by a narrow sinuous passage that runs through distal end of pouch. Vas deferens between internal vesicle and cirrus describes an elongate, transversely arranged, figure-of-eight loop. Cirrus proper, 280 × 20 μ, with thick but seemingly nonmuscular wall; apparently only slightly protrusible—only in occasional segments does it project beyond the margin; armed with very fine hair-like spines and provided with a cirrus whip, a very long, slender, hollow filament which arises near the base of the cirrus and extends through its lumen and well beyond its tip—some 300 μ long, the whip appears to be chitinous and somewhat stiff although the free end is variously looped and coiled about.

Host: *Anas acuta tzitzihoa* Vieillot, 1816 = *Dafila a. t.* (V.).

Habitat: Lumen of small intestine.

Locality: Stillwater, Payne County, Oklahoma.

Cotype specimens: Several complete strobilae mounted on separate slides, in U. S. Nat. Mus. Helm. Coll., No. 36807.

According to Hughes (1941) the genus *Hymenolepis* Weinland, 1858, contains some 328 recognized-as-valid species and varieties. The new form was studied in comparison with descriptive data (mostly from Hughes, 1941) on all of the previously known species. So far as the writer has been able to determine only eleven other members (enumerated below) of the genus have been described as having a structure comparable to the cirrus whip. Except as otherwise indicated the original descriptions of these species were used in the present study. Differing from *Hymenolepis mastigopraedita* n. sp. with regard to features severally mentioned:

(1) *H. collaris* (Batsch, 1786) Fuhrmann, 1908, as described by Krabbe (1869) under the name of *Taenia sinuosa* (Zeder, 1800) Rudolphi, 1810, and by Joyeux and Baer (1936), has the testes very considerably smaller, the cirrus proper more protrusible, and the cirrus whip heavier and less protrusible;

(2) *H. columbae* (Zeder, 1800) Railliet and Henry, 1909, as described by Fuhrmann (1906) and Southwell (1930) under the name, *H. sphenocephala* (Rudolphi, 1810) Fuhrmann, 1906, and by Joyeux and Baer (1936), has a shorter cirrus pouch, characteristic angles in the

course of the vagina, the sacculus accessorius farther posteriad with respect to the cirrus pouch, and the cirrus stylet apparently not protrusible;

(3) *H. cyrtoides* (Mayhew, 1925) Fuhrmann, 1932, is of much smaller size and has an unarmed cirrus and no sacculus accessorius;

(4) *H. flamingo* Skrjabin, 1914, is much smaller and has the cirrus pouch and stylet both much shorter and broader and the genital atrium surrounded by a chitinous ring;

(5) *H. krabbeella* Hughes, 1940, as described by Fuhrmann (1906) and Joyeux and Baer (1936) under the name, *H. fragilis* (Krabbe, 1869) Fuhrmann, 1906, has the testes arranged in a straight line and the cirrus pouch twice as long as the width of the proglottid;

(6) *H. lintoni* López-Neyra, 1932, as described by Linton (1927) under the name, *H. "macracanthos* (Linstow)", apparently has no sacculus accessorius;

(7) *H. longistylosa* Tseng Shen, 1932, has the testes arranged in a transverse row, a very small cirrus pouch, the cirrus whip many times as long as the width of the proglottid, and no sacculus accessorius;

(8) *H. macracanthos* (von Linstow, 1877) Fuhrmann, 1906, has a 3-lobed ovary, no sacculus accessorius, and the cirrus pouch much longer than the width of the proglottid according to Fuhrmann (1924);

(9) *H. serrata* Fuhrmann, 1906, and the variety,

(10) *H. serrata birmanica* Meggitt, 1924, have the testes arranged in a straight line; and

(11) *H. venusta* (Rosseter, 1897) Railliet and Henry, 1909, has a much shorter cirrus pouch, the internal seminal vesicle folded back and forth in a characteristic series of appressed loops, and the testes disposed in a transverse row according to Rosseter (1898).

The occurrence of the pseudoscolex in the new species would seem to be very unusual if not unique within the genus. In each of the enumerated forms the scolex is known and armed with 8 or 10 rostellar hooks. *H. flamingo* was taken from *Phoenicopterus antiquorum; H. columbae, H. serrata,* and *H. s. birmanica* are parasitic in columbiform birds; all of the other worms nominately mentioned above were described from anseriform hosts.

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EXPLANATION OF PLATE

All figures concern *Hymenolepis mastigopraedita* n. sp.

ABBREVIATIONS

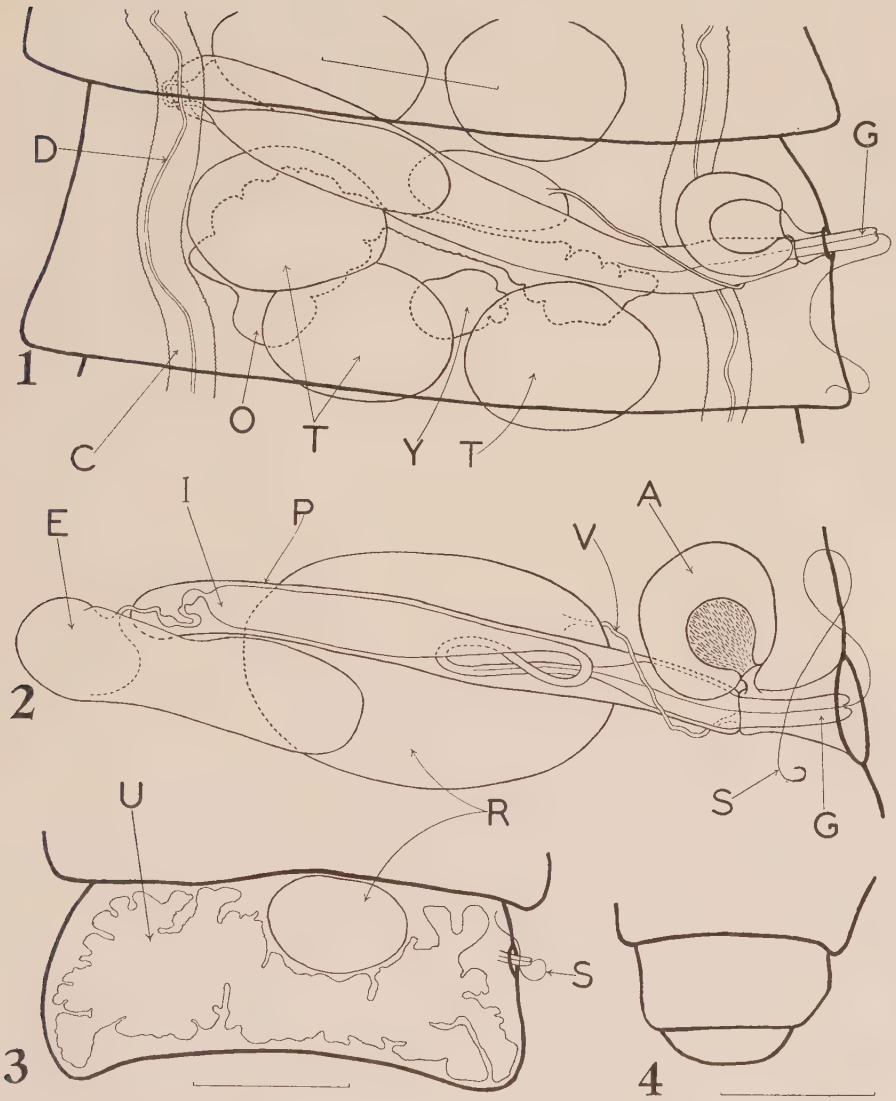
A—sacculus accessorius
C—ventral longitudinal excretory canal
D—dorsal longitudinal excretory canal
E—external seminal vesicle
G—cirrus
I—internal seminal vesicle
O—ovary
P—cirrus pouch
R—seminal receptacle
S—cirrus whip or stylet
T—testes
U—uterus
V—vagina
Y—vitellarium

FIG. 1. Mature proglottid. Dorsal view. Camera lucida study. Scale, 250 μ.

FIG. 2. Freehand study of genital tracts in a partially gravid segment. Dorsal view. The spines in the sacculus accessorius are more numerous, more elongate, and more hair-like than the figure suggests.

FIG. 3. A gravid segment drawn with the aid of a projection apparatus. Scale, 500 μ.

FIG. 4. Terminal segments of a strobila, drawn with the aid of a projection apparatus. Scale, 1 mm.



Hymenolepis mastigopraedita n. sp.

HAEMOGREGARINA TRIATOMAE N. SP. FROM A SOUTH AMERICAN LIZARD *TUPINAMBIS TEGUIXIN* TRANSMITTED BY THE REDUVIID *TRIATOMA RUBROVARIA*[1]

J. J. Osimani[2]

Talice in 1929, examining the rectal contents of the blood-sucking reduviid *Triatoma rubrovaria* (Blanchard 1843) coming from the Cerro de Montevideo, noticed "croissant"-like cells which he considered as sporozoites of a haemogregarine. In 1939 we made the same finding in several nymphs and adults of *Triatoma rubrovaria* coming from Maldonado, which also contained *T. cruzi*. If they were, indeed, the final sporogonic forms from a haemogregarine, what was their vertebrate host? The problem, as we see, was reversed from the way it commonly develops with haemogregarines whose species have been described in great number only in the vertebrate. In this case, we knew the intermediate host; the question was to find the definitive host. Talice had suggested that the definitive host might possibly be the small lizard *Teious teyou,* in whose burrows *Triatoma rubrovaria* frequently dwells. The blood examination of six specimens of that lizard, coming from that place, proved negative in 1939. However, later on, in 1940, examining a young lizard which came from Maldonado, we were successful in finding it parasitized with haemogregarines. The lizard specimen was classified by the learned Director of the Natural History Museum of Montevideo, Dr. G. Devincenzi, as *Tupinambis teguixin* L.

In the blood films stained by Pappenheim's method, the parasites showed the following appearance: worm-like shaped cells, slightly curved, measuring 16 μ in length and 5.5 μ in width, with rounded and very similar ends. In some examples a kind of fine, long fissure, parallel to the longer axis of the protozoon, which sometimes penetrates very deeply in the body (Figs. 6 and 8) is seen at one end. This does not seem to be an artifact but rather the folding of the parasite within its capsule, a phenomenon which is known in many haemogregarines. There is a prolonged nucleus, smaller than half the length of the haemogregarine; the longer axis parallel to the longer axis of the latter. It stains violet and is placed near the convex side of the parasite, near the boundary between the middle third and the terminal third.

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[1] From the Division of Parasitology (Prof. R. V. Talice, Chief) of the Institute of Hygiene of Montevideo (Prof. A. N. Berta, Director).

[2] I wish to express appreciation to Dr. Clay G. Huff for help received in the preparation of this manuscript for publication.

It is evident from examples 6 and 8, if we admit the explanation given above, that the relations of the position and length of the nucleus would be different. The cytoplasm is of a light blue color without metachromatic granules. It appears in nearly all the examples surrounded by a colorless halo, doubtless a real capsule which impedes sometimes the penetration of the stain, as it happens in Figs. 2 and 3, in which the nucleus is not visible.[3] The longer axis of the parasite is parallel to the longer axis of the red cell, which is generally increased, showing very often a hyper- or hypochromatic cytoplasm. The nucleus of the red cell, altered sometimes in its tinctorial reactions, is pushed back to the ends or to the sides of the cell.

On the membrane of the parasite is sometimes noticed a small red spot (Fig. 4) analogous to one noted by Reichenow in *H. stepanowi* and which he considers to be a residue of volutine.

The characteristics previously described belong, no doubt, to gametocytes. We looked for the schizogonic forms in the sections of several internal organs stained by eosin-haematoxylin. After a long search which we had already foreseen owing to the very small number of blood parasite cells, we found in the liver a division cyst. It is an elliptical cyst (Fig. 10), measuring 20 μ in length and 14 μ in width, with a double wall, the internal one being fine and smooth and separated from the external by a fine colorless halo, the external wall being thick (it measures 2 μ on its thickest side), of irregular surface and staining violet by haematoxylin. However, it is possible that what looks like an external wall may really be calcium deposited around the cyst. To determine this point, it would be necessary to stain by special methods. The cyst seems to be confined within an epithelial cell whose nucleus is greatly misshapen.

Coming back to the starting point, is this haemogregarine, found in *Tupinambis teguixin* (and over whose classification we do not ponder for the time being), identical with that found spontaneously in the "vinchuca"[4] *T. rubrovaria?*

Availing ourselves of "vinchucas" from the colony kept in our Institute for making xenodiagnosis of Chagas' disease, we fed nymphs and larvae of these laboratory-bred *T. rubrovaria* on infected lizards. After the blood meal these insects were kept at a temperature of 30° C for two months and then examined.

In many of them we limited the examination to the rectal contents, for it was there that we had found the spontaneous infections. On others we made total dissections of the abdominal contents, studying the midgut, the Malpighian tubes, the genital organs, and the general cavity. Of 30

[3] However, the hypothesis that they might be empty capsules, abandoned by the parasite, cannot be dismissed.

[4] Common name for the blood-sucking Reduviidae in Uruguay and Argentina.

FIGS. 1–21. Reference line, 20 microns.

FIGS. 1–9. Intracorpuscular forms from the blood of the lizard, *Tupinambis teguixin.*

FIG. 10. Schizogonic cyst in the liver of *Tupinambis teguixin.*

FIGS. 11–13. Sporogonic forms in the general cavity of *Triatoma rubrovaria;* in 13 is already seen the segmentation of the oöcyst.

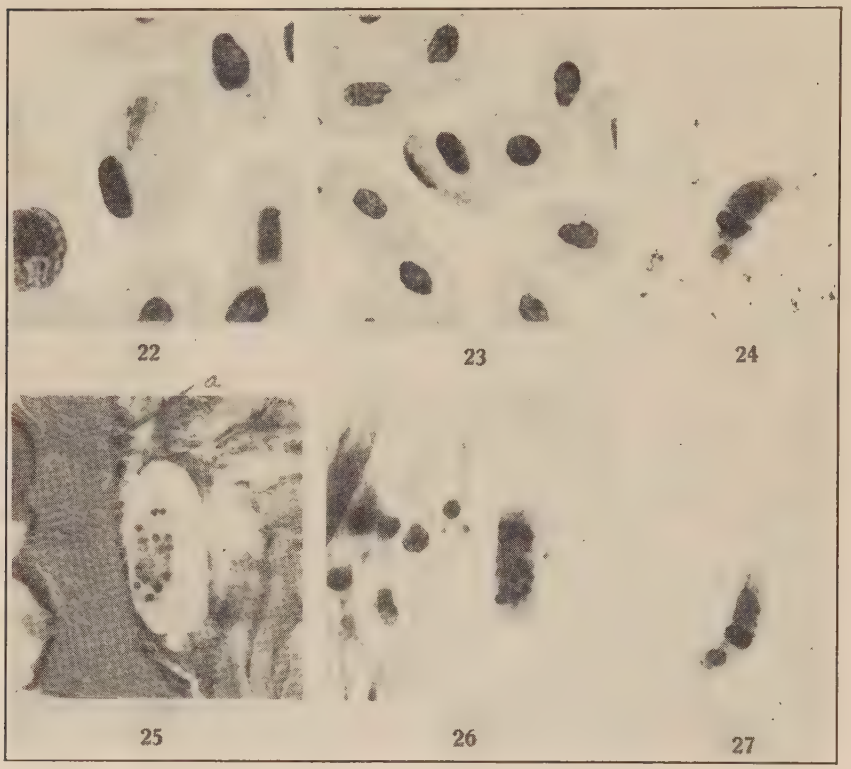
FIGS. 14–18. Sporozoites from the rectum of *Triatoma rubrovaria,* spontaneous infection.

FIGS. 19–21. Sporozoites from the rectum of *Triatoma rubrovaria,* experimental infection.

examples fed upon *Tupinambis teguixin,* only one was positive.[5] The percentage of infection would be only 3.3% in this experiment, but the

[5] In more than 500 "vinchucas" used in the Laboratory for making xenodiagnosis of Chagas' disease, these parasites have never been found.

limited blood infection of the vertebrate must be taken into consideration. First we shall describe the cells found in the "vinchucas" with spontaneous infections, and afterwards those examined in the experimental infection of *T. teguixin.*



FIGS. 22–23. Photomicrograph of red cells from *Tupinambis teguixin* parasitized by *Haemogregarina triatomae.* ×1000.

FIG. 24. Photomicrograph of sporozoite from the rectum of *Triatoma rubrovaria.* ×650.

FIGS. 25–26. Photomicrograph of the formation of the oöcyst in the general cavity of *Triatoma rubrovaria; a.* cuticle of the insect. ×500.

FIG. 27. Photomicrograph of the sporozoite from the rectum of *Triatoma rubrovaria.* ×650.

SPONTANEOUS INFECTION OF THE INTERMEDIATE HOST

Examination of the rectal contents showed two kinds of organisms: free sporozoites and cysts. The cysts, elliptical in shape, contain a great number of sporozoites which are clearly seen through the transparency of their smooth and fine wall. The cysts measure from 60 μ to 80 μ in length and 42 μ to 48 μ in breadth. They contain from 20 to 40 sporozoites. The unstained sporozoites look like crescents, with a sharper end, a

rounded and clearly visible central or sub-central nucleus and a granulated cytoplasm. Stained by the Pappenheim's method (Figs. 14 to 18) the nucleus appears misshapen; the reticulated cytoplasm shows condensations on both sides of the nucleus. Its length varies between 18 μ and 30 μ, its breadth between 4 μ and 7 μ. The examination of the Malpighian tubes and the genital organs was negative, nor could we find the enormous cysts of the intestinal cell wall which Talice mentions in his report. The morphological characters of these sporozoites are more or less analogous to those mentioned by Talice.

EXPERIMENTAL INFECTION OF THE INTERMEDIATE HOST

In the experimental material we found only cysts. They are elliptical in shape, with a smooth and fine wall through which the sporozoites can be seen (about 30). The sporozoites are left free by the flattening and breaking of the cysts. These, stained by the Pappenheim's method (Figs. 19 to 21), have an appearance similar to those in the spontaneous infections, but with a larger nucleus and a cytoplasm which is different at the two ends, and without condensations. They measure from 20 μ to 25 μ in length and 5 μ to 6 μ in breadth.

On examining sections of insects in paraffin, we looked for early stages of the sexual phase. In this way we noticed that during the growth of the protoplasmic mass, originated perhaps by the conjugation of the gametes, its nucleus begins to divide (Fig. 11), the protoplasmic mass grows longer and the nucleus migrates to the periphery (Fig. 12). In Fig. 13 the sporozoites have begun to develop in the ends. Later on, all the protoplasmic mass changes into sporozoites which line themselves up parallel to the longer axis of the cyst. In this way the cyst is formed.

We cannot state in which of the insect organs such development takes place. We exclude, as explained above, the Malpighian tubes and the genital organs. We have not found parasites in the intestinal wall. The forms which we described (Figs. 11 to 13) were found in the general cavity. Relying only upon the examination of the insect, everything suggests (similar host, localization, and morphology) that the haemogregarine found in spontaneous infection of *T. rubrovaria* by Talice (1929) and me in 1939, and that found in the experimental infection are a single species. The slight morphological dissimilarities between the sporozoites might be explained by the fact that we were dealing with matured sporozoites in the spontaneous infection while those we saw in the experimental infection would not yet be mature. Thus the haemogregarine observed in the lizard, *T. teguixin,* undergoes its sporogonic cycle in the "vinchuca," *T. rubrovaria,* terminating in the formation of sporozoites.

The infectious nature of these sporozoites, however, will not be established until the inoculation of lizards with parasitized bugs has been

attained. Since uninfected lizards were not available at the time we did our experiments, the cycle remains uncompleted.

IDENTIFICATION

"The numerous species of haemogregarines described are nearly all distinguished from one another only by their different hosts, and only in a few cases is our knowledge about them enlarged with the description of their phases of development" (Reichenow, 1920). Certainly the characters which are used for identifying the haemogregarines (except the host species) are generally morphological differences, sometimes rather undefined, which may correspond to different stages or forms of the parasite (micro- or macrogametocyte, young or adult gametocyte). So it frequently happens that it is difficult to affirm whether or not a given form belongs to a species previously described. By now four species of haemogregarines have been described from *Tupinambis teguixin.* These are *H. tupinambisi* Laveran and Salimbeni, 1909; *H. carini* (Laveran) 1909; *H. missoni* Carini, 1909; and *H. iguanae* Ducceschi, 1914. Plimmer (1912) limits himself to mentioning a haemogregarine discovered by him in *T. teguixin* without giving any description.

In the inclosed table the characters of these four species may be compared with those of the species discovered by us. The differences among *Haemogregarina carini, missoni,* and *iguanae* are very clear. In the adult form of *H. tupinambisi* there are more analogies, but these analogies do not suffice for identifying them. Writers speak about the great volume increase of the parasitized red cells, which attain 25 μ to 28 μ. In our case the red cells never reach 20 μ. *H. tupinambisi* shows red compact granules, while we did not notice any. The nucleus of *H. tupinambisi* is central and oval in shape; that of our haemogregarine is never in the center, but between the center and one of the ends; besides, it is genuinely prolonged.

We firmly believe then that we have before us a new species, a schizogonic cyst of which we have found in the liver of the lizard and several sporogonic forms of which we have found in the blood sucking reduviid, *T. rubrovaria.* We propose for it the name *Haemogregarina triatomae.*

Haemogregarina triatomae n. sp.

Hosts: Vertebrate: South American lizard, *Tupinambis teguixin.* Invertebrate: *Triatoma rubrovaria.*

Morphology: Erythrocytic forms (gametocytes) worm-like, slightly curved, 16 μ long, 5.5 μ wide, ends very similar. A long fissure parallel to longer axis appears in some, apparently a folding of parasite on itself. Nucleus prolonged, smaller than half the parasite and parallel to length of parasite, violet (with Pappenheim's stain). Cytoplasm light blue, no metachromatic granules, surrounded by colorless halo or capsule. Red cell generally increased in length by parasite which lies lengthwise in it. Cytoplasm of host cell may be hypo- or hyperchromatic. Host nucleus sometimes altered in staining reaction and pushed aside or to end of cell. Small red spot on membrane of parasite.

TABLE 1.—*Haemogregarines of the lizard* Tupinambis teguixin

	H. carini	*H. missoni*	*H. tupinambisi*	*H. iguanae*	*H. triatomae*
Protoplasmic alteration of the red cell	None	None	Great volume increase, reaching 25μ to 28μ; misshapen form, light lilac color	None	Generally an increase of volume; hypo- or hyperchromatic cytoplasm
Nuclear alteration of the red cell	Sometimes peripheral	Normal position	Misshapen, hypertrophied, leaning towards one side or towards the end; sometimes divided in two parts	Scarce	Rejected towards one side or towards the end; sometimes modified in its tinctorial reactions
Shape of the parasite	Vermicular	Kidney-like	Cylindrical	Kidney-like	Vermicular, slightly curved, some specimens seem folded on themselves
Size	15–18μ × 3–4μ	8–9μ × 2.5–3μ	14–16μ × 5–6μ	13.5–15.5μ × 3.5–5μ	16μ × 5.5μ
Capsule			Surrounded by colorless halo		Surrounded by colorless halo
Granules	Fine and red, with deep staining		Thick and red		None
Position of the nucleus	In the center	Central	Central, with its longest axis perpendicular to that of the parasite		At junction of middle third and terminal third; close to the convex side of the parasite; longest axis parallel to that of the parasite
Shape of the nucleus	Prolonged, with very irregular outlines	Oval or round	Oval	Prolonged of irregular outlines	Prolonged
Size of the nucleus	7–12μ; ½ or ⅔ of length of parasite			Much larger than one-half of the parasite's length	Much less than one-half of the parasite's length
Other forms observed in the blood	Forms with queerly rounded ends (femen.?), with light cytoplasm and oval nucleus	None	Young forms of 10μ × 2.5μ; one end thick and round, the other fine and a little curved. The cytoplasm with fine granules and the central nucleus prolonged with its axis parallel to that of the parasite. Without capsule. Red cell slightly altered with nucleus not disjointed	Young forms (queer) smaller, of 6μ and 4μ	None
Schizogony	Not found	Not found	Not found	Not found	Division cyst in the liver
Sporogony	Not found	Not found	Not found	Not found	In *Triatoma rubrovaria*

Schizogonic stages: In liver of *T. teguixin,* elliptical, 20 μ long, 14 μ wide, with double wall, inner one fine and smooth, external one thick (maximum 2 μ), irregular, violet (by haematoxylin). Cyst apparently in epithelial cell with misshapen nucleus.

Cysts: In *Triatoma rubrovaria,* elliptical, 60–80 μ long, 42–48 μ wide, with large number of sporozoites (20 to 40).

Sporozoites: Crescent-shaped, 18–30 μ long, 4–7 μ wide, one end sharper, rounded central or subcentral nucleus, granulated cytoplasm; when stained they show condensations on both sides of nucleus. In sporogony nucleus divides while protoplasmic mass is enlarging, then migrates to periphery. Sporozoites form and line up parallel to long axis of cyst.

Type locality: Maldonado.

Type host: Tupinambis teguixin.

SUMMARY

A new species, *Haemogregarina triatomae,* in the lizard *T. teguixin* L. is described. The infection was scanty; we succeeded in finding, besides the blood stages, a division cyst in the liver. Of 30 "vinchucas" from laboratory-bred *Triatoma rubrovaria* fed upon the infected lizard, one showed different sporogonic forms, including sporozoites, in its tissues and in its rectal contents. These sporozoites are similar to those found by Talice (1929) and by the author (1939) in the rectal contents of "vinchucas" of the same species spontaneously infected.

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AGE RESISTANCE OF MICE TO *TRYPANOSOMA CRUZI*

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The trypanosome of Chagas' disease, *Trypanosoma cruzi,* is known to cause more severe infections in young persons than in adults, and when the organism is transferred to laboratory rats, mice, and rabbits, young animals likewise usually suffer more intense infections than older individuals (1–4). The guinea pig, on the contrary, is about equally susceptible to the parasite at all ages (3).

Although mouse infections with *Trypanosoma cruzi* have been studied by several investigators, the precise age at which mice attain their comparative resistance is still uncertain since the specific ages of the animals used in the studies reported hitherto has not generally been known. In the present work, an attempt has been made to fix this age for mice, by studying carefully the relative susceptibility of mice of different known age groups. The infections were carried out both by intraperitoneal inoculation of the parasites and by their administration per os.

MATERIALS

The strain of *Trypanosoma cruzi* was obtained originally from the London School of Hygiene and Tropical Medicine in 1936, and has since been kept in this laboratory in the rat or in culture. For administration in the present work, cultures were prepared by adding 0.1 cc of infected rat heart's blood to 5 cc of nutrient broth. The cultures were used after from four to six weeks, when many organisms had developed.

The mice were of the Swiss strain, propagated in the Department of Animal Care of Columbia University.

THE COURSE OF INFECTION IN MICE GIVEN *T. cruzi* INTRAPERITONEALLY

Mice ranging in age from newly-born to 100-day old animals were infected intraperitoneally with doses of *T. cruzi* culture in proportion to body weight, 0.1 cc of culture being given per 10 gm weight. At intervals of 1 or 2 days thereafter, the tail blood of the mice was examined for the presence of trypanosomes, the total number in 50 microscope fields (×430) being recorded. The data on representative mice from a total of 77 animals are given in Table 1.

Young mice suffered distinctly heavier infections with the parasite than older mice. Animals below 25 days old when infected invariably succumbed (47 animals), whereas those above this age usually survived.

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TABLE 1.—*Resistance of representative mice of different age groups to* Trypanosoma cruzi *administered intraperitoneally*

Mouse No.	Age when infected (days)	Total number of parasites in 50 fields (×430) on designated day after infection														
		9	10	12	14	16	18	20	22	24	26	28	30	32	34	36
1	Newborn	0	2	5	12	236	[d]									
2		1	5	3	36	165	865	[d]								
3	5	0	0	1	1	6	150	[d]								
4		0	0	0	1	8	12	[d]								
5	8	0	0	3	1	3	16	[d]								
6		0	0	5	9	6	23	[d]								
7	10	0	2	16	140	[d]										
8		0	1	6	1	70	[d]									
9	14	0	0	0	0	1	0	1	5	18	10	[d]				
10		0	0	0	0	0	0	0	10	35	34	[d]				
11	20	0	0	1	1	7	3	1	18	[d]						
12		0	0	0	3	0	6	28	[d]							
13	25	0	0	0	1	21	3	17	15	86	36	56	65	2	2	0
14		0	0	2	2	6	15	24	[d]							
15	33	0	0	0	0	0	0	0	1	0	2	3	2	1	0	0
16		0	0	0	0	0	2	0	0	5	0	6	2	2	0	0
17	46	0	0	0	0	0	0	1	0	0	1	0	1	1	0	0
18		0	0	0	1	0	0	1	0	2	7	7	8	5	4	0
19	74	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0
20		0	0	0	0	0	0	0	0	0	2	1	0	0	0	0
21	100	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
22		0	0	0	0	0	0	0	1	0	0	0	0	0	0	0

[d] = dead.

The prepatent period of the infection in animals below 25 days of age at infection also was shorter than that of the mice above this age, its length for the two groups being, respectively, 7 to 12 days, and 14 to 26 days.

THE COURSE OF INFECTION IN MICE GIVEN *T. cruzi* BY MOUTH

An amount of the trypanosome culture roughly proportional to the body weight was administered by mouth to a total of 74 mice of different age groups. Great care was exercised to avoid trauma in administering the dose, a droplet of the culture merely being placed in the opened mouth of each animal. Control mice of each age group were infected at the same time intraperitoneally with 0.1 cc of culture per 10 gm body weight.

TABLE 2.—*Relative susceptibility of mice of different age groups to infection after per os administration of* Trypanosoma cruzi

Age group (days)	Number in group		Number positive		Percentage positive	
	By mouth	I–P	By mouth	I–P	By mouth	I–P
Newborn	8	2	8	2	100	100
2	9	2	9	2	100	100
6	7	2	7	2	100	100
9	6	2	3	2	50	100
12	13	2	2	2	15	100
16	11	2	3	2	27	100
20	6	2	0	2	0	100
25	7	2	0	2	0	100
40	4	2	0	2	0	100
85	3	1	0	1	0	100

At intervals of 1 or 2 days thereafter, the tail blood of the animals was examined microscopically (×430) for trypanosomes. The relative susceptibility of the mice, in terms of the number and percentage infected in each age group, after per os and after intraperitoneal administration of the parasites is shown in Table 2. The relative intensity of infection in representative mice after administration of the parasite by the two methods is shown in Table 3.

TABLE 3.—*Comparative intensity of representative infection after per os and after intraperitoneal administration of* Trypanosoma cruzi

Mouse No.	Age when infected (days)	Route of infection	Number of parasites in 50 fields (×430) on designated days after infection											
			8	10	12	14	16	18	20	22	24	26	28	30	32
1	Newborn	Per os	0	0	0	0	0	0	1	4	30	34	16	3	0
2		" "	0	0	0	0	0	0	0	1	1	4	0	0	0
3		" "	0	1	36	270	[d]								
4		I–P	1	5	84	310	[d]								
5		Per os	0	0	0	0	0	0	1	1	0	0	1	0	0
6	3	" "	0	0	0	0	0	1	0	0	2	1	3	3	0
7		I–P	0	0	3	7	13	24	[d]						
8		Per os	0	0	0	0	1	4	2	5	1	7	2	0	0
9	9	" "	0	0	12	33	3	3	2	5	2	2	27	46	[d]
10		I–P	0	2	4	4	[d]								
11	11	Per os	0	0	3	3	1	3	2	1	2	0	0	0	0
12		I–P	0	0	8	27	[d]								
13	15	Per os	0	0	0	0	0	1	0	0	0	0	0	0	0
14		I–P	0	2	1	0	1	2	8	[d]					

[d] = dead.

Mice of groups below 9 days of age when exposed to per os infection were invariably susceptible to *Trypanosoma cruzi,* the parasites being observed later in their tail blood. Among the mice of groups from 9 to 16 days old at exposure, only 50 per cent or less became infected. Animals 20 days old or more when exposed were regularly refractory to infection by organisms given per os. In contrast, all the control animals infected intraperitoneally with the culture showed the parasite later in the blood stream.

The intensity of infection among the control group injected intraperitoneally was distinctly greater than that of animals which received the organisms by mouth. Most of those which developed an infection after the per os administration survived the disease, whereas the intraperitoneally inoculated controls for these animals invariably succumbed. Usually, the prepatent period after the administration of organisms by mouth was longer than that after their intraperitoneal injection.

The results offered from the present study in mice are essentially confirmatory of those previously published from this laboratory upon age resistance of rats to *Trypanosoma cruzi* (4). Very young mice, like the young rats, evidently are unable to check infections with this trypanosome and soon succumb to it, whereas older animals seem to overcome the parasite with little trouble.

The infections in mice with this strain of *Trypanosoma cruzi* appear usually to be of distinctly lower intensity than those in rats. Often, in fact, the infections even in young mice are so poor as to seem inconsequential until a day or so before the host's death, when many organisms come suddenly into the peripheral blood. Generally, even so, the peak intensity of the mouse infections is considerably below that of rats of corresponding age. This difference may result from the fact, previously stated, that the strain of *Trypanosoma cruzi* used has been kept for several years in the albino rat.

The susceptibility of mice to infection by mouth is likewise essentially the same as that of the rat (5). The less intense infections of mice exposed by mouth compared with those injected intraperitoneally is probably explained by the smaller number of parasites which succeed in entering the animals after administration by mouth. Invasion by fewer parasites prolongs the prepatent period and increases the possibility for the host to check the invader. When the prepatent period is short, as it sometimes is after per os administration, however, the infection usually progresses to the fatal end expected after intraperitoneal administration of the parasite.

SUMMARY

Mice below 25 days of age when infected generally succumb to *Trypanosoma cruzi* administered intraperitoneally, whereas mice above this age usually survive the infection. The infections in young animals develop after a shorter prepatent period and attain greater intensity than those in older animals.

Mice below 9 days of age can regularly be infected with *Trypanosoma cruzi* administered by mouth, and those from 9 to 16 days old often prove susceptible. Mice 20 days old or more resist the parasite given orally. Infections resulting from oral administration of *Trypanosoma cruzi* are generally less intense and less frequently fatal than infections following the intraperitoneal administration of the parasite.

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NEMATODIRUS TORTUOSUS N. SP. (NEMATODA) FROM THE RAT, *NEOTOMA*

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During the summer of 1940 nematodes of the genus *Nematodirus* Ransom (1907) were obtained from the small intestines of two species of rats of the genus *Neotoma.* The rodents were trapped in the vicinity of the campus of the University of California at Los Angeles. In May five females and one male were taken from a *Neotoma lepida intermedia;* in July two females and 18 males were found in a *N. fuscipes macrotis.* Since morphological studies showed that these worms represented a species as yet undescribed, the name *Nematodirus tortuosus* is proposed for them.

Nematodirus tortuosus n. sp.
(Figs. 1–6)

Diagnosis: Nematodirus.

Male: 16.00–16.80 mm long, maximum diameter 125 μ. Cephalic cuticular dilation 125 μ long by 60 μ wide, with transverse striations. Body with longitudinal cuticular ridges throughout. Cervical papillae absent. Esophagus 470 μ long. Excretory pore 485 μ from anterior end. Bursa outspread, 500 μ wide. Two large lateral, two small dorsal lobes. Rays long, delicate. Externodorsal thin, 155 μ long; dorsal ray bifurcated in distal 2/7, lateral raylet being longer and itself bidigitate. Margin of bursa distal to small, numerous bosses corrugated by fine ridges about 2.5 μ apart running out to and causing a serrated bursal border. Spicules 2.38–2.90 mm long, equal or unequal in length, yellow-brown in color, with transverse striations. Free proximal parts, measuring about 420–440 μ long by 15 μ in diameter; fused distally forming a single structure with a maximum diameter of 22 μ. Finger-like tip contained in a loose membranous bulb. Pre-bursal papillae absent.

Female: 23.50–28.75 mm long, maximum diameter 245 μ at vulva. Cephalic dilation 130 μ long by 65 μ wide, transversely striated. Anterior two-thirds of body with, posterior third without longitudinal cuticular ridges. Esophagus 500 μ long. Excretory pore 525 μ from anterior end. Vulva 7.35 mm from posterior extremity. Vagina a transverse slit about 60 μ long. Ovejectors very long, combined length including sphincters being 1.55–1.60 mm. Moderate diminution in body size behind vulva to 200–250 μ from posterior end where diameter decreases abruptly to truncated tail. Tail 75 μ long with bristle-like process 13 μ long. Ova smooth-shelled, oval, measure 150–178 by 75–88 μ.

Hosts: Neotoma lepida intermedia and *Neotoma fuscipes macrotis.*

Location: Small intestine.

Locality: West Los Angeles, California.

Type specimens: U.S.N.M. No. 36717; *paratypes* No. 36718. Other paratypes in the Department of Zoology, University of California at Los Angeles.

As far as can be determined, the genus *Nematodirus* Ransom (1907) was last revised in 1937 by Travassos. In his opinion the genus included

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16 species (although he lists 17). His list is: *Nematodirus filicollis, N. spathiger, N. weinbergi, N. roscidus, N. mauritanicus, N. neotoma, N. molini, N. abnormalis, N. helvetianus, N. dromedarii, N. tarandi, N. leporis, N. mugosaricus, N. aspinosus, N. oiratianus, N. triangularis, N. urichi.*

In regard to the relationship of *N. tortuosus* to the other members of the genus, we find it must be classified as a form occupying a position between the *filicollis* and *neotoma* groups (May, 1920). The first of these was characterized by comparatively short spicules and the location of the vulva near the junction of the middle and posterior thirds of the body. The *neotoma* group, conversely, had relatively long spicules; the vulva was set well back. Members of both groups had divergent ovejectors and uteri.

N. tortuosus occupies an intermediate position. The vulva in the *filicollis* group is placed so that the ratio of total body length to the tail-vulva distance is 3 : 1. *N. neotoma* has the vulva at or slightly in front of the middle of the posterior third of the body, 5 or 6 : 1. *N. tortuosus* has a ratio of 3.6 : 1. From this position the uteri diverge, one passing into the anterior, the other into the posterior part of the body.

The ratio of spicule- to body-length likewise illustrates the point. For *N. filicollis* this is approximately 1 : 12, for *N. neotoma* 1 : 3. *N. tortuosus* has a ratio of 1 : 6.

N. tortuosus is most closely related to *N. aspinosus* Schulz, 1929 (= *N. neomexicanus*), and to *N. leporis* Chandler, 1924, both of which have been found in rodents. *N. aspinosus* resembles it in body length and in general bodily proportions such as the distances from the tail to vulva and anus. The spicules are comparable in that they are unequal and connected throughout most of their extent, but they are less than half as long as those of *N. tortuosus,* the ratio of spicule- to body-length being only about 1 : 16. The female differs in having no process on the tail and in the greater size of the ova (209–215 by 116–123 μ).

N. leporis is a smaller form measuring 8.0–13.0 mm for males and 16.5–20.0 mm for the length of females. The location of the vulva, the size of the ova, the general character of the bursa, are much the same as in *N. tortuosus.* The spicules of *N. leporis* however are relatively much shorter (0.65–1.05 mm) than those of *N. tortuosus.* On the other hand, the transverse striations on their unequal proximal parts and their fusion into a single structure which ends in a finger-like tip makes them resemble those of *N. tortuosus* more than those of any other species.

The relation of *N. tortuosus* to *N. neotoma* is of particular interest because both have been found only in rats of the genus *Neotoma. Nematodirus neotoma* Hall, 1916, has been taken from *Neotoma desertorum, N. fallax, N. baileyi,* and from *N. rupicola; Nematodirus tortuosus* from

two different species: *Neotoma lepida intermedia* and *N. fuscipes macrotis*. The morphology of the nematodes likewise definitely sets them apart: *N. neotoma* is a smaller form with males measuring 8.0–10.5, females 18.3–22.2 mm. The spicules are proportionately longer than those of *N. tortuosus*. The ratio of the posterior extremity-anus distance to total body length is 1 : 170 as compared to 1 : 350 for *N. tortuosus* and the vulva has a posterior position which serves to differentiate the females of the two species.

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Explanation of Plates

Nematodirus tortuosus n. sp.

All figures were drawn with the aid of a camera lucida.

Plate I

Fig. 1. Anterior end of male, lateral view.
Fig. 2. Posterior extremity of female, showing spinous process.
Fig. 3. Region of vulva, showing vagina, ovejectors, ova, beginning of uteri.

Plate II

Fig. 4. Spicules: a–dorsal view of tips. b–lateral view of tips; c–cross section of fused portion.
Fig. 5. Spicules: a–entire spicules, lateral view; b–proximal unequal portions.
Fig. 6. Bursa, dorsal view.

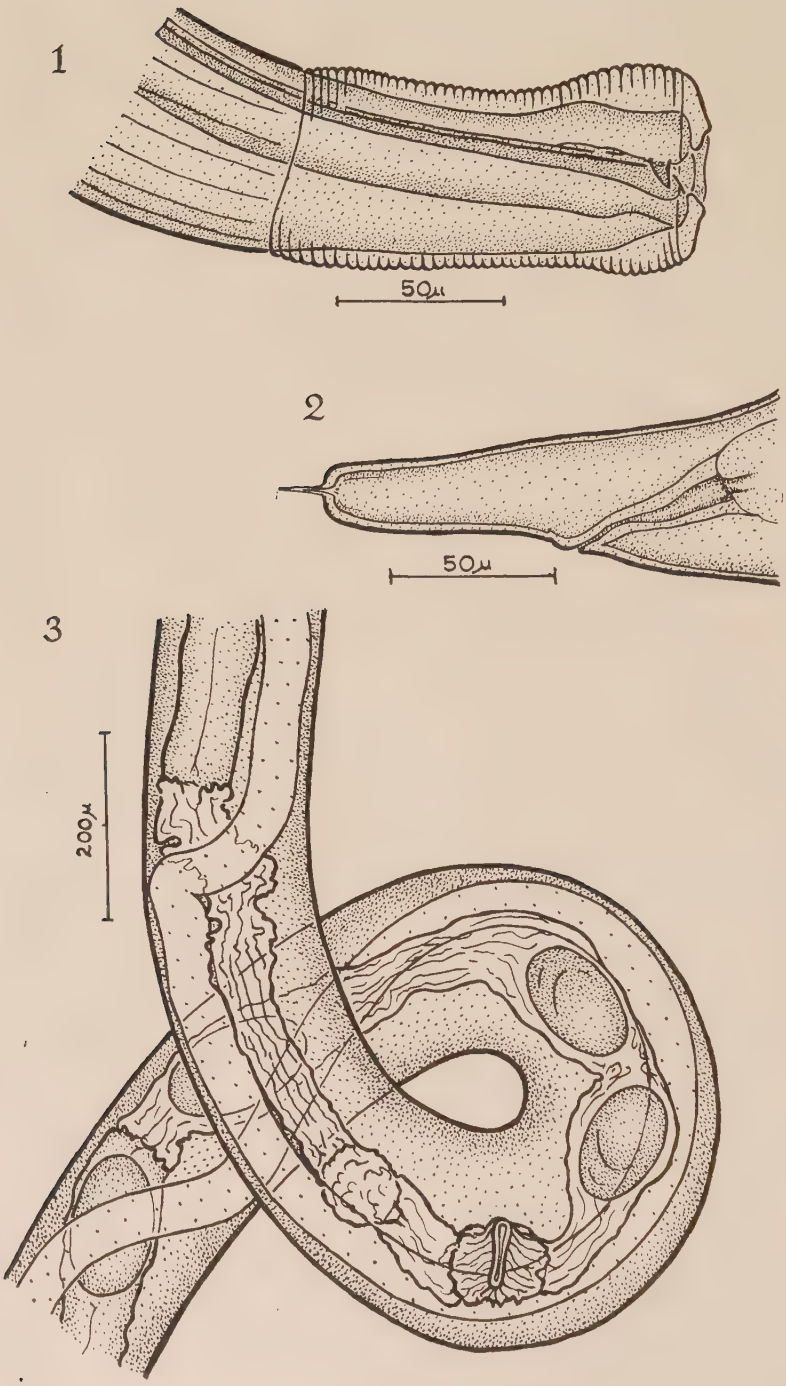


PLATE I

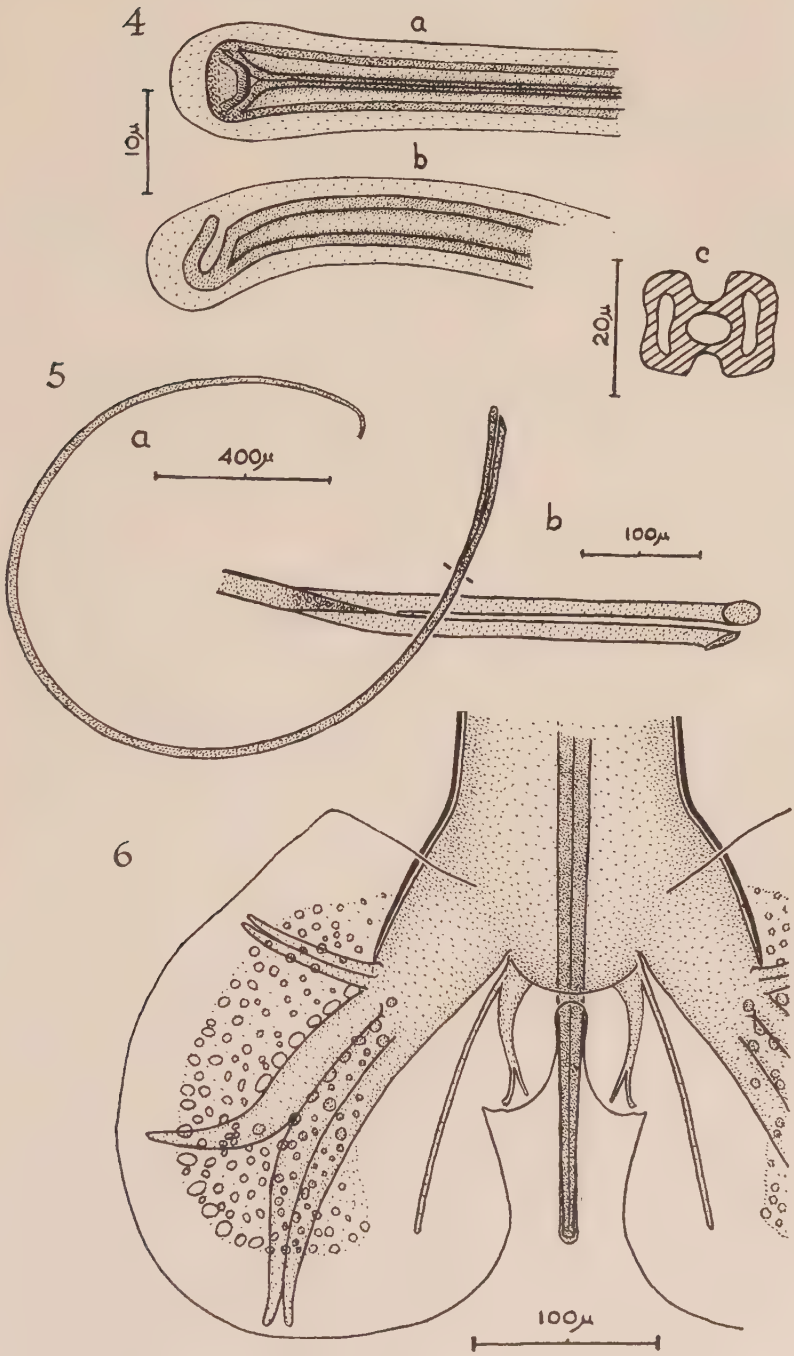


PLATE II

RESEARCH NOTES

NOTE ON THE ASSOCIATION OF THE TICK, *ORNITHODOROS TALAJE* (GUÉRIN-MENEVILLE), WITH BAT INFESTATIONS IN HUMAN DWELLINGS IN BRAZIL

Specimens of a tick were collected by the author in 1939 at Carmo do Rio Claro, Minas Geraes, Brazil, on the ceiling of a farmhouse infested by many bats, and were identified as *O. talaje.* Ticks of the same species from Campo Belo, a neighboring country of the above, had later been sent to Dr. H. Beaurepaire Aragão at the Instituto Oswaldo Cruz by Dr. A. Botelhos, who found them in beds in a house in that area.

In addition, there were frequent reports in Carmo do Rio Claro of a tick found on the ceiling of houses, which was increasing in numbers, and whose bite caused edema and severe itching in persons. The possibility that flies of the families Nycteribiidae and Strebliidae, common in this region, were responsible for the bites was discounted when inhabitants of the farmhouses failed to describe them. In addition, these flies are not known to leave their bat hosts.

Large numbers of the bat, *Histiotus velatus* Geoff., were present in 2 farmhouses, and adult and 2 nymphal ticks were captured in a corner where the bats rested during the day.

Specimens of this species were also taken in the bedroom of still another farmhouse. Apparently the ticks fall into the beds from the ceilings which, in old farmhouses of this region of Brazil, are loosely constructed of bamboo. The fact that only larvae or nymphs parasitize the man is probably explained on the basis of a narrower host range in the adult.

Recently (1940) in Viçosa, the author received specimens of this tick collected in a house by the inhabitants who stated that ticks frequently fell from the ceiling. The ticks disappeared when the bats were removed. Bats, therefore, appear to be responsible for the introduction of these pests.

According to Aragão (1936, Mem. Inst. Oswaldo Cruz **31**: 759–844), *O. talaje* is common in holes of the rodent, *Kerodon rupestris.* Larvae and nymphs have been collected on *Coelogenes paca* and *Dicotyles tajacu.* That author states that large numbers of that species were found in a cave in Matto Grosso, Brazil, by Dr. Alipio de Miranda Ribeiro who made no reference to bats in the cave, however.

So far as the author is aware, this is the first reference to the occurrence of the tick, *O. talaje,* as parasites of bats inside dwellings. In his opinion, the adult ticks in such places are strictly parasites of bats and removal of the bats also eliminates the ticks.—José Candido M. Carvalho, *Escola Superior de Agricultura e Veterinaria, Viçosa, Brazil; and University of Nebraska.*

PARALAEURIS CUCKLERI N. SP. (NEMATODA) FROM THE IGUANA (*CYCLURA CORNUTA*)

In 1938, A. C. Cuckler reported on the nematodes parasitic in a land iguana (*Conolophus subcristatus*) from the Galapagos Islands (Allan Hancock Pacific Expeditions, **2**: 137–165. U. of S. Calif. Press) and established a new genus of oxyurid worms—*Paralaeuris* (Syphaciinae)—with *P. dorochila* as the type species. The present writer recently had the opportunity of examining a number of parasites collected from a West Indian iguana (*Cyclura cornuta*) which had died in the Zoological Gardens at Washington, D. C. Among the collections were large numbers of both sexes of an oxyurid that not only established the validity of the genus *Paralaeuris,* but added a second species to that genus. The new species is designated as *Paralaeuris cuckleri,* in recognition of the work done by Cuckler on the parasites of the Iguanidae.

Paralaeuris cuckleri n. sp.

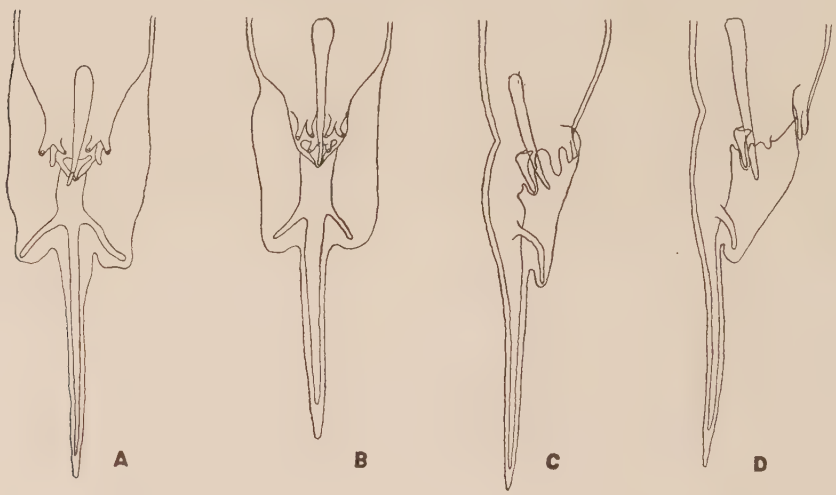
Males: Average of 10 specimens. Length, 2.5 mm; greatest width, 0.125 mm; pharynx length, 0.018 mm; esophagus length, 0.5 mm; bulb length, 0.063 mm; head-nerve ring distance, 0.148 mm; head-excretory pore distance, 0.66 mm; cloaca-tail distance, 0.18 mm; spicule length, 0.081 mm; accessory piece length, 0.018 mm; caudal papillae, two pairs of precloacal pedunculate papillae (almost double papillae), one pair of sessile papillae on the anterior lip of the cloaca, and one pair of pedunculate postcloacal papillae. Pedunculate papillae support distinct caudal alae.

Females: Average of 10 specimens. Length, 3.1 mm; width at vulva, 0.19 mm; pharynx length, 0.018 mm; esophagus length, 0.5 mm; bulb length, 0.063 mm; head-nerve ring distance, 0.15 mm; head-excretory pore distance, 0.75 mm; vulva-tail distance, 1.5 mm; anus-tail distance, 0.72 mm; egg size, 0.027×0.042 mm. Eggs are unsegmented at time of oviposition.

Type host: West Indian Iguana (*Cyclura cornuta*).

Habitat: Posterior intestine of host.

The lips of both sexes are provided with cuticular projections, as in the case of *P. dorochila* Cuckler, and the excretory pore is similarly post-bulbar in position. The vagina and ovejector are well-developed. *P. cuckleri* may be distinguished from



Ventral and lateral views of the tail region of males of *Paralaeuris cuckleri* and *P. dorochila* to show difference in size and arrangement of caudal structures.

A. *Paralaeuris dorochila.* Ventral view of tail of male.
B. *P. cuckleri.* Ventral view of tail of male.
C. *P. dorochila.* Lateral view of tail of male.
D. *P. cuckleri.* Lateral view of tail of male.

P. dorochila on the bases of (1) the larger size of the male and the corresponding increase in the size of the other male structures, (2) the difference in the structure and arrangement of the caudal papillae of the male, (3) the median position of the female vulvar opening, (4) the smaller size of the eggs, and (5) particularly by the fact that the posterior portion of the esophagus in both sexes is glandular instead of muscular as in *P. dorochila.* All of these points have been checked against cotype material of *P. dorochila.*

The material studied was made available through the courtesy of the Zoological Division of the Bureau of Animal Industry, U. S. Department of Agriculture, and the type material is deposited in their collections.—A. C. Walton, *Knox College, Galesburg, Illinois. Contribution No. 77.*

A *PARAGORDIUS* LARVA (GORDIACEA) IN A TREMATODE

A single *Paragordius* larva was found in the parenchyma of a trematode taken from the small intestine of the hog molly, *Hypentelium nigricans* collected from Honey Creek near Ann Arbor, Michigan, on May 3, 1941. The trematode was identified as being very closely related to *Plagioporus sinitsini* Mueller, 1934 (Allocreadiidae). One of six trematodes contained, just anterior to the genital pore, a *Paragordius* larva in a very early stage of development. The larva was free in the parenchymatous tissue. No spontaneous movement was visible and there was no indication of a cyst. The larva was very loosely bent into the shape of a letter J, the posterior end being curved. This is the second record of the occurrence of a larva of Gordiacea in a trematode. Cort (1915, J. Parasitol. **1**: 198–199) found *Gordius* larvae in the trematode species, *Brachycoelium hospitale* Stafford, 1903, from the intestines of the green newt, *Diemictylus viridescens*. He found eight of sixteen trematodes from several different hosts infected, two containing two larvae and the others one each.—Jacob H. Fischthal, *Dept. of Zoology, University of Michigan, Ann Arbor, Mich.*

EIMERIA ANTELOCAPRAE, A NEW COCCIDIUM FROM THE AMERICAN ANTELOPE

A young antelope was struck by an automobile some twenty or more miles north of Laramie, Wyoming, February 9, 1941. Authorities of the Wyoming Game and Fish Commission were notified, and the badly hurt animal was killed and brought to the parasitology laboratory at the University of Wyoming stock farm. An examination for parasites was made the following day. A study of the contents of the cecum and large intestine revealed numerous oöcysts.

Two antelope fecal samples collected February 19, 1941, at a spring hole some twenty miles north of Laramie contained the same type of oöcysts as those found in the killed animal. Of seven samples collected in the same area on March 19, three contained the oöcysts, but in these they were not numerous.

To the writer's knowledge the literature includes no record of coccidia from the American antelope. The oöcysts are not like those reported from cattle and sheep (Becker, 1934, "Coccidia and Coccidiosis"; Christensen, 1938, Proc. Helm. Soc. Washington **5**: 24; Christensen, 1938, J. Parasitol. **24**: 453–467; Christensen and Porter, 1939, Proc. Helm. Soc. Washington **6**: 45–48) although sheep and cattle range over the same area. Furthermore, the oöcysts are different from those described from *Orias canna* (Triffitt, 1924), *Capella rupicapra, Cervus axis, Cervus canadensis, Cervus capreolus, Cervus elaphus, Dama dama,* (Rastegaieff, 1930, Arch. Protistenk. **71**: 377–404), and *Gazella subgutturosa* (Yakimoff et al, 1932, Z. Parasitenk. **5**: 85–93). These oöcysts are, therefore, believed to represent a new coccidium for which the name *Eimeria antelocaprae* is proposed.

The length and width measurements given in the description of these oöcysts are based on a study of 200 oöcysts: 67 from the killed animal, and 67 from one and 66 from the other of the two fecal samples collected February 19. The differences among the length means and width means of the three groups are not statistically significant. The means and standard deviations for the three groups and for the total 200 oöcysts are as follows:

	Group 1	Group 2	Group 3	Total
Number	67	67	66	200
Mean length μ	31.1	31.2	30.2	30.8
St. dev.	2.0	2.5	2.3	2.3
Mean width μ	26.5	26.4	25.3	26.0
St. dev.	1.2	1.7	1.4	1.5

The sporont range in size and mean as given is based on measurements from 25 oöcysts.

Eimeria antelocaprae n. sp.

Figs. 1 and 2

Description of oöcysts: Oöcysts 24.6 to 35.2 μ (average 30.8 μ) long by 21.1 to 29.5 μ (average 26.0 μ) in transdiameter. Shape typically ellipsoidal, from 1.04 to

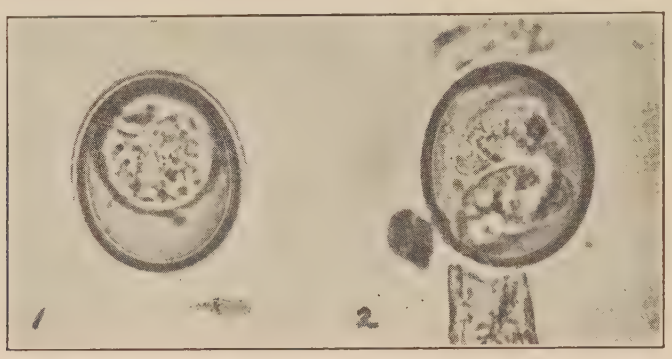


Fig. 1. Unsporulated oöcyst of *Eimeria antelocaprae* n. sp.
Fig. 2. Sporulated oöcyst of *Eimeria antelocaprae* n. sp.

1.34 (average 1.18) times as long as broad. Wall approximately 2.2 μ thick, smooth, transparent, nearly colorless to a faint dull yellow-green in tint with that part of wall just outside oöcyst membrane darker. No micropyle nor thinning of wall at one end apparent. Sporont 16.5 to 19.8 μ (average 17.8 μ) in diameter. Spores approximately 16.5 long by 9.2 μ in transdiameter. Oöcystic residual material present as a few small irregularly shaped particles.

Type host: Antelope (*Antelocapra americana*)

Type locality: Near Laramie, Wyoming.

—Henry Huizinga, *Agricultural Experiment Station, University of Wyoming, Laramie.*

STUDIES UPON *STRONGYLUS VULGARIS*

VI. Tests with Organic Copper Salts

Continuation of in vitro tests upon *Strongylus vulgaris* similar to those reported previously (Whitlock, 1940, J. Parasitol. **26**: 45–47; 49–57) has revealed several facts which may be of importance in vivo work with anthelmintics.

The methods used in these tests have been described in detail in the previous papers. Briefly, a population of female *Strongylus vulgaris* was collected from infected ceca at a nearby horse-slaughtering establishment. Test groups were selected at random from this population and exposed to the action of the proper copper salt in warm 0.85% NaCl solution for 100 minutes. Each test contained one control group exposed to 0.85% NaCl alone. At intervals thereafter the worms were tested for the presence of rigor mortis with a special apparatus. Those showing rigidity to resist a perpendicular pressure of 4.4 grams were considered dead.

The chemicals used were CP. Molarity as herein used means the amount of salt per unit of water, not necessarily in solution.

Table 1 gives the results of this series of experiments in which the measurement of efficacy of the concentration of various copper salts used is given by the LT50 (time necessary to kill one-half of the test group). This was estimated as in previous papers by means of graphic analysis of probit mortality. It may be noted that the same concentration of each salt used produces a markedly different effect on different sample populations, but that salts which are constant in solubility produce almost the same relative effect on any given sample population as the standard salt

copper sulphate, a fact noted before in experiments with the cupric halides (Whitlock, op. cit.).

Some of the salts, however, present points of special interest. Copper tartrate, even though relatively insoluble, is effective in destroying *Strongylus vulgaris* in vitro. This is not true of many insoluble inorganic compounds, such as copper arsenite and carbonate. This accounts for copper tartrate's efficacy in destroying nodular worms, as reported by Monnig (1935, Onderstepoort J. Vet. Sc. **5**: 419–438). Copper formate is also worthy of notice since it is so much more effective than copper sulphate and since the same concentration of sodium bicarbonate (0.1 per cent) which inactivates 0.01 molar solutions of inorganic copper compounds does not completely immobilize it.

TABLE 1

Experiment number	$CuSO_4$	Copper acetate	Copper ⊙ tartrate	Copper sulphocarbolate	Copper formate	Copper formate + .1% $NaHCO_3$
1	596		645		206	429
2	689	524*	740	454	298	
3	746	714	613	568		
4	562		552		182	
5	1208×				196	460

* Basic copper acetate—has twice as much copper available as ordinary copper acetate.

⊙ Solubility is variable.

× Probably an error.

Note: Each experiment contained one control group whose LT50 was at least three times larger than the largest LT50 in the experiment except in experiment 5.

Three conclusions may be drawn from these data. (1) This paper reemphasizes the existence of a marked secular variation in the reaction of *Strongylus vulgaris* to a lethal agent. Such a marked secular variation must be guarded against in tests in vivo. (2) Organic copper salts, unlike inorganic, need not be water soluble to be effective against a parasite either in vivo or in vitro. (3) Copper formate, because of its markedly lethal action and because of its resistance to inactivation by sodium bicarbonate, should be tried as a substitute for copper sulphate in such diseases as haemonchosis of sheep.—J. H. WHITLOCK, *Kansas Agricultural Experiment Station, Contribution No. 89, Department of Veterinary Medicine.*

INTESTINAL PARASITES IN BOYS OF THE FLORIDA INDUSTRIAL SCHOOL

A survey of 188 white inmates of the Florida Industrial School for Boys (delinquent juveniles), situated in Jackson County, Florida, has been made recently to determine the incidence of helminthic and protozoan parasites. Jackson County in 1937–38 was shown (Leathers, Keller, and McPhaul, 1938, Am. J. Hyg. **87**(D): 1–16) to have a hookworm incidence of 44 per cent. Recently gathered unpublished data have shown this county to have an incidence of 60 per cent.

The individuals examined in this survey have come from nearly every county in the state of Florida and represent a high school age group, i.e., 12 to 19 years inclusive.

A single examination of each specimen was performed by each of the three following methods: (1) Within a few hours after passage of the specimen a fecal smear for iron hematoxylin staining was made. (2) All specimens were then examined by both the simplified zinc sulfate centrifugal floatation technic, and (3) the Willis' brine levitation technic. The zinc sulfate in this simplified technic had a specific gravity of 1.2, the same as the salt, and the cheesecloth straining and second washing were omitted.

Intestinal parasites were found in 122 individuals (65 per cent). Nine different species of parasites were recorded. Forty-four (23 per cent) harbored helminths and 107 (57 per cent) were found infected with protozoa.

The simplified zinc sulfate centrifugal floatation technic followed by staining with D'Antoni's iodine was found to be the best general procedure for the recovery of helminth ova and protozoan cysts. Thirty-five (75 per cent) of the helminth infections, 77 (72 per cent) of the protozoan infections, and 94 (77 per cent) of the total parasitic infections were revealed by this method.

TABLE 1

Postives found	Positives found by all three methods		Most efficient method	Per cent positives found by most efficient method
	No.	Per cent		
Hookworm	35	19%	Brine	14%
Whipworm	9	5%	Zinc sulfate	4%
Tapeworm (*H. nana*)	1	0.5%	Zinc sulfate	0.5%
Endamoeba coli	81	43%	Zinc sulfate	34%
E. histolytica	8	4%	Iron H.	3.7%
Endolimax nana	29	15%	Iron H.	10%
Giardia lamblia	18	9%	Zinc sulfate	7%
Iodamoeba bütschlii	6	3%	Iron H.	3%
Chilomastix mesnili	2	2%	Iron H.	2%
Total helminths	44	23%	Zinc sulfate	18%
Total protozoa	107	57%	Zinc sulfate	41%
Total parasite incidence	122	65%	Zinc sulfate	50%

The incidence of helminthic infections was lower than that known for most of the state of Florida and only about one-third that for the county in which the school is located. However, conditions prevailing in this institution would tend to prevent the spread of hookworm infection and this in turn would lower the helminth incidence. Furthermore, hookworm infections in new arrivals at the school are treated. Most cases found in this study were probably residual infections from inefficiently treated cases.

Protozoan infections were three times more numerous in this group than has been noted in the general population in Florida (unpublished studies). It is not known whether the individuals examined obtained their protozoan infections before or after entering the school. Food handlers in this institution were found uninfected.—WILLIAM A. SUMMERS, *Senior Parasitologist, Bureau of Laboratories, Florida State Board of Health, Jacksonville, Florida.*

A NOTE ON VITAMINS IN RELATION TO ECTOPARASITE RESISTANCE

Searls and Snyder (1935, J. Econom. Entom. **28**: 304–310) conducted some experiments on the control of ectoparasites of laboratory rats in 1935. During the course of the work it was noticed that those animals in better health apparently had less lice than those in a poor condition. These incidental observations were repeated many times and it was suggested that perhaps dietary factors had something to do with the condition. Vitamin A was thought of first since there is abundant evidence to show that this factor has a profound effect upon endoparasites and is a physiological prophylactic against infection, in general.

During the years 1936–37 the author conducted experiments at the University of Wisconsin on the relation of vitamin A to resistance of the albino rat to its lice. The results at that time indicated a strong positive correlation between adequate amounts of vitamin A in the diet and resistance of the laboratory rat to the multiplication of the rat louse, *Polyplax spinulosa* Burm.

Searls and Snyder (1939, J. Parasitol. **25**: 425–430), basing further experimental work on the preliminary results of Kartman, concluded that "vitamin A was the main factor which regulated the resistance or susceptibility of the rats to the lice."

Further observations upon the problem substantially confirm the preliminary results of Kartman and the work of Searls and Snyder. At this time it is possible

to add actual louse counts taken post mortem which give a quantitative value to the gross observations of louse infestation on the live rats. Rats maintained at a level of avitaminosis-A showed an average infestation of 1451.4 lice per rat; rats given the curative treatment for avitaminosis-A showed an average infestation of 28.2 lice per rat; the control rats showed an average infestation of 12.5 lice per rat. These data are taken only from those rats which lived for 25 days or more after the onset of avitaminosis-A. It was also concluded that neither sex nor litter had any significant effect upon immunity to lice in the rat host.

Notwithstanding the positive evidence cited above, the statement of Searls and Snyder attributing to vitamin A the main limiting role in the ability of the rat to resist the rat louse may be too sweeping, and the problem may not be quite that simple and clear-cut. Gyorgy (1938, Proc. Soc. Exper. Biol. and Med. **38**: 383–385) showed that pediculosis was apparent in about 20 per cent of rats kept on a riboflavin-free diet for from eight to ten weeks. The lice were almost completely eliminated from the host by the therapeutic feeding of riboflavin. Asa C. Chandler (1941, Farrar and Rinehart, N. Y.) in his recent book on nutrition, "The Eater's Digest," after pointing out that members of the vitamin B_2 complex may be linked with sulphur metabolism, suggests that this may be the way in which riboflavin influences pediculosis.

In any event, it seems clear at this time, that we cannot state that any particular vitamin plays the single or even the "main" limiting role in determining the resistance of rats to their lice. The known work on this problem to date has shown simply that two variates (amount of vitamin in the diet and resistance to lice) maintain a fairly definite relation to each other. However, they tell nothing about the cause of the relationship but merely reveal its existence.

The actual data therefore suggest that vitamin A cannot be taken as the "main factor" in the resistance of albino rats to lice. Rather it is one nutritional variate having a significant relation to possible cutaneous or metabolic changes which may cause a true immunity to develop against lice in the rat host.—Leo Kartman, *U. S. Department of Agriculture, Bureau of Entomology and Plant Quarantine.*

COMMUNICATIONS

CARLOS FINLAY AND YELLOW FEVER

To the Editorial Committee of the JOURNAL OF PARASITOLOGY:

I am most appreciative of the lengthy and detailed review of my book "Carlos Finlay and Yellow Fever" published in the August 1941 Journal by Dr. F. L. Soper, and I am in complete accord with his statement that:

> "It is indeed regrettable that unpleasant discussions as to who should get the credit for the mosquito transmission of yellow fever have occurred; surely there was glory and to spare for all who had any part in contributing to the work which led to the control of the most fearful of tropical scourges."

the last part of which reproduces almost word for word the remarks of Dr. Guiteras at the banquet, presided over by Gen. Wood in Havana on December 22, 1900. As well as with that:

> "Surely it will not be difficult to reconcile rival claims."

My father and I have always been the first to recognize to the full the brilliant confirmatory and complementary work of the Reed Commission, permitting myself to reproduce the following words of my father at the above-mentioned banquet:

> "Twenty years ago, guided by indications, which I deemed certain, I sallied forth into an arid and unknown field; I discovered therein a stone, rough in appearance. I picked it up and with the assistance of my efficient co-laborer —Dr. Claudio Delgado—polished and examined it carefully, arriving at the conclusion that we had discovered a rough diamond. But nobody would believe us, till twenty years later, when there arrived a commission composed of intelligent men, who in a short time extracted from the rough shell the stone to whose brilliancy none can now be blind."

I must however take exception to some remarks of Dr. Soper somewhat in contradiction with his conciliatory phrases.

In the first place I object to my book being considered a brief at all and much less as a brief for the plaintiff in the case of Finlay vs. Reed et al, and Gorgas. In any case it might be considered as a brief for the defense against persistent and unwarranted non-recognition of the full scope of my father's work. It is moreover misleading, in this regard, to mix the Reed Commission with Gorgas, as my father never had any issue with the latter, whose share was limited to the sanitary practical confirmation of the mosquito theory, independent of the individual share of the parties concerned.

The issue at the stake between my father and the Commission is the non-admission by the latter of the former's alleged "positive cases" of experimental yellow fever, admittedly fewer in proportion and milder in intensity to theirs, based solely on the duration of the "extrinsic incubation," since they never discussed the cases clinically, an argument possibly tenable at a time when the supposed yellow fever parasite was held, like that of malaria, to spend a phase of its existence in the body of the mosquito, but is no longer so after the brilliant work of the South American yellow fever workers (including Dr. Soper) which shows that the virus in the mosquito is infective from the moment of its ingestion and that the length of time required for it to reach its salivary glands and be infective through the bite varies from four days onwards according to the outside temperature.

I deplore with Dr. Soper the introduction of nationalistic arguments into this discussion, a fault to be found with some participants on both sides of the controversy.

The relative merits of the parties concerned are most clearly set forth in the following documents of strictly North American origin:

I. Gorgas' final Report to Gen. Wood (Report of Brig. Gen. Wood—Report of Chief Sanitary Officer, July 12, 1902; reproduced on page 118 of my book):

"In the summer of 1900 the commission of Army and medical officers headed by Walter Reed, U. S. Army, had been sent to Cuba for the investigation and study of yellow fever. Due to the financial assistance given by the Military Governor to this commission they were enabled to experiment on human subjects. They took up the theory advanced by Dr. Carlos J. Finlay of Havana in the year 1881 that the stegomyia mosquito was the sole means of transmission of yellow fever. Dr. Finlay had maintained his theory for some twenty years and had done considerable experimental work in this direction.

The commission, through elaborate and careful experiments proved this to be correct. . . . I myself had seen the work and was convinced that the mosquito could convey yellow fever but I was hardly prepared to believe that it was the only way or even the ordinary way of conveying the disease."

II. Memorial Tablet presented to the City of Havana by the City of New Orleans on December 3, 1941:

Mayoralty of the City of
New Orleans, Louisiana, U.S.A.

To His Honor, the Mayor of the
City of Havana, Cuba.
Greetings:

The people of New Orleans, recognizing their unpayable debt to *Carlos Finlay,* the immortal discoverer of the transmission and propagation of yellow fever by the mosquito; and,

Remembering that it was by the adoption of Carlos Finlay's principles of anti-mosquito sanitation, so decisively confirmed by the heroic labors of the United States Army Yellow Fever Commission under the direction of Walter Reed, and irrefutably demonstrated by the marvelous efficiency of the anti-mosquito campaign conducted by Colonel William C. Gorgas in Havana in 1899–1900, that Havana and Cuba were definitely and permanently rid of the yellow fever pestilence; and,

Since New Orleans, profiting by the example of Havana by adopting the same anti-mosquito tactics and strategy, achieved a definite and decisive victory over yellow fever in 1905, which delivered not only New Orleans but the whole North American continent from the terrors of the Yellow Fever peril, and again,

In view of the unparallelled era of salubrity, prosperity and civic improvement that has followed in the wake of that epochal victory of sanitary science over yellow fever in our city in 1905; and,

Furthermore, in view of the happy and constantly growing commercial, cultural and good neighborly relations between New Orleans and Havana, that has been made possible primarily through Finlay's discovery, it is hereby

Resolved and ordered by the City Council of New Orleans that a copy of this letter be transmitted in suitable form for permanent preservation, to the Mayor of the City of Havana as a testimonial of gratitude and appreciation contributed by the City of New Orleans to the public celebration of the one hundred and eighth anniversary (108th) of the birth of Carlos Finlay which is held in Havana on December 3rd, 1941, and that our honored and beloved fellow citizen, Dr. Rudolph Matas, Commander of the Cuban Order of Carlos J. Finlay, be commissioned to make this presentation in behalf of the City of New Orleans.

The Mayor of the City
of New Orleans.

Yours very sincerely,
C. E. Finlay, M.D.

Vedado, Habana.

CONSERVATION OF SCHOLARLY JOURNALS

To the Editorial Committee of the JOURNAL OF PARASITOLOGY:

We are enclosing a brief statement of one of the aims of the Committee on Aid to Libraries in War Areas.

> The American Library Association created this last year the Committee on Aid to Libraries in War Areas, headed by John R. Russell, the Librarian of the University of Rochester. The Committee is faced with numerous serious problems and hopes that American scholars and scientists will be of considerable aid in the solution of one of these problems.
>
> One of the most difficult tasks in library reconstruction after the first World War was that of completing foreign institutional sets of American scholarly, scientific, and technical periodicals. The attempt to avoid a duplication of that situation is now the concern of the Committee.
>
> Many sets of journals will be broken by the financial inability of the institutions to renew subscriptions. As far as possible they will be completed from a stock of periodicals being purchased by the Committee. Many more will have been broken through mail difficulties and loss of shipments, while still other sets will have disappeared in the destruction of libraries. The size of the eventual demand is impossible to estimate, but requests received by the Committee already give evidence that it will be enormous.
>
> With an imminent paper shortage attempts are being made to collect old periodicals for pulp. Fearing this possible reduction in the already limited supply of scholarly and scientific journals, the Committee hopes to enlist the cooperation of subscribers to this journal in preventing the sacrifice of this type of material to the pulp demand. It is scarcely necessary to mention the appreciation of foreign institutions and scholars for this activity.
>
> Questions concerning the project or concerning the value of particular periodicals to the project should be directed to Wayne M. Hartwell, Executive Assistant to the Committee on Aid to Libraries in War Areas, Rush Rhees Library, University of Rochester, Rochester, New York.

The Committee will be grateful for your cooperation in this attempt to notify individual scholars of its hopes and plans.

Sincerely,

WAYNE M. HARTWELL,
Executive Assistant